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Importance of major gene effects on quantitative traits in the fowl

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Importance of major gene effects on quantitative traits in the fowl

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INTRODUCTION

Modern methods of animal and poultry breeding rely mainly on the principles of quantitative inheritance for the improvement of commercial strains. For about three decades (1900-1930) many breeders were highly optimistic that major gene effects with typical mendelian segregation would be discovered for the important economic traits. For the most part, however, this has not been realized; instead, greater refinements in quantitative genetics, such as selection indices, have been used.

Breeders are again studying "mendelizing" traits in the hope that this approach will supplement methods of improvement in current use. Several developments have contributed to this renewed interest in mendelian genetics. Some traits, such as egg production in chickens, seem to be reaching a plateau, so that further improvement by conventional quantitative methods may be slow and inefficient. The trait early feathering has at least one major sex-linked locus with alleles K, k; knowledge of this has made it possible to develop quickly early feathered broiler strains. Also, this locus, being sex-linked, can be used to determine the sex of chicks at an early age.

Certain major gene differences are in part responsible for susceptibility to virus infection according to recent work on lymphoid leukosis and Marek's disease (Vogt, 1967;

Stone, 1967; Stone et al., 1970). Many biochemical polymorphisms, such as blood groups, serum allotypes, esterases, and transferrins, in a wide assortment of species, have been discovered. Some blood group genes, such as B^1 in chickens, are clearly important in viability. Much greater laying house mortality has been observed for B^1B^1 homozygous hens over that for other genotypes (Rishell, 1968). The question is, how generally important are other biochemical variants in determining differences in egg production and other reproductive fitness traits. Also, certain morphological mutants, including dwarfism in cattle and chickens, may be economically important.

Two general types of major genes were considered in this study: those affecting morphological variation and those affecting biochemical variation. The morphological trait studied was sex-linked dwarfism and the biochemical characters were allotypic differences in serum proteins. Sex-linked dwarfism is produced by a recessive gene, dw. The gene causes a marked reduction in body weight and bone length (Hutt, 1959). Allotypes are antigenic specificities on serum proteins which differ among individuals within a species.

Practical utilization of major gene effects may first require the development of experimental populations. In this study, two cross-line dwarf populations and a single

population segregating for allotype alleles were developed. Such populations are useful for studies of single locus genotypes on egg production, body weight, egg weight, and other performance traits.

The main objectives of this study were: (1) to study the effect of the dwarf gene on performance traits in chickens, (2) to combine three allotype subpopulations into a single population segregating only for known allotype alleles, and (3) to reproduce the standard allotype antisera developed earlier by David (1966). A secondary objective was to find a more efficient method for making allotype antisera in chickens. An attempt was also made to gain information on: (a) whether allotype antisera in chickens can be produced from donors and recipients differing by only one known allele, (b) the influence of donor and recipient family relationship on anti-allotype precipitin production, (c) the influence of donor and recipient origin on response to antigenic stimulation, and (d) the effect of antigen dose and frequency of injection on antibody response.

PART I. IMMUNOGLOBULIN ALLOTYPES IN THE FOWL

REVIEW OF LITERATURE

Allotypes are genetically determined antigenic specificities, present on serum proteins, which differ between individuals within the same species. Several classes of immunoglobulins exist in different species of animals. Immunoglobulins belonging to classes IgG, IgA, and IgM are common to several species, including humans, mice, rabbits, and chickens. Immunoglobulin molecules are made up of two light polypeptides chains (molecular weight of about 20,000 each) and two heavy polypeptide chains (molecular weight of approximately 50,000 each, Fleischman et al., 1962). Digestion of these molecules with papain yields three subunits: two Fab subunits and an Fc subunit (Porter, 1959). Fc contains only heavy chains, while Fab contains both heavy (Fd) and light chains. A segment of each light chain and part of the Fd portion of the heavy chains have variable amino acid composition while the remainder of the molecule has a relatively constant amino acid composition. Allotypic determinants have been detected on both the Fc and Fab subunits.

Schutze (1902) was first to report isoprecipitins to serum components in rabbits. More recently, serum isoprecipitins were produced in rabbits by injecting recipients with ovalbumin - anti-ovalbumin in paraffin oil (Oudin, 1956). Oudin (1956) proposed the term "allotypy" to denote the distinct antigenic variants of serum proteins against which

the antibodies in the isoimmune sera were directed. Since that time, many allotypes have been detected in several species of animals.

In general, allotypes are denoted by a capital A followed by a number (Dray et al., 1962). A lower case letter after the A indicates the genetic locus involved; thus Aa1 is allotype A1 at locus a.

Allotypes in Chickens

Serum isoantigens in chickens were first reported by Skalba (1964a). Hens were injected intravenously with Proteus vulgaris. The anti-bacterial sera were then used to agglutinate the bacteria, and the antigen-antibody complex was injected intravenously into another group of hens three times per week for seven to ten months. Three allotypes, GA1, GA2, and GA3, were identified by Ouchterlony's precipitation test in gel. Skalba (1964b, 1964c, 1966) subsequently identified nine additional specificities, GA4 through GA12. Allotypes GA4, GA5, GA6, and GA8 were gamma globulins, as determined by electrophoretic mobility, while the remainder migrated as beta globulins. Allotypes GA3, GA5, GA6, and GA8 were found in egg yolk proteins as well as in serum.

The most extensive studies on allotypes in chickens have been carried out by David (1966) and David et al. (1969). Donor chickens were first immunized against Brucella abortus

and the resulting antisera were then used to agglutinate the bacteria. The agglutinates, containing specific antibody, were emulsified in Freund's incomplete adjuvant and injected by the subcutaneous route. Recipient sera were then tested by Ouchterlony's double diffusion in gel method (Ouchterlony, 1953). Nineteen different allotypic specificities were detected, eight of which have shown genetic segregation. The allotypes were evidently controlled by codominant alleles Aa^1 and Aa^2 at locus a, Ab^1 and Ab^2 at locus b, and Ac^1, Ac^2, Ac^3, and Ac^4 at locus c. The specificities determined by the a locus were apparently located on IgG molecules. The Ab1 allotype has also been detected in egg yolk protein. Whether or not the allotypes of Skalba (1966) and David (1966) represent the same or different specificities is unknown.

Natural antiglobulin systems were first detected in chicken sera by David et al. (1965). Natural antibodies to serum groups seem to be quite common in various breeds and crosses of chickens (Petrovsky et al, 1966; McDermid et al, 1969).

The frequencies of six allotypes were surveyed in five lines of White Leghorn chickens selected for metric traits by David et al. (1966). Allotypic distribution of reactions showed wide variation between lines. Since all lines were derived from the same base population, this suggested that

allotypic frequencies were influenced by selection.

Certain allotypes in chickens may disappear with the onset of lymphoid tumor growth (David and Fletcher, 1970.) Thirty White Leghorn chickens were typed for allotypic determinants before and after injection with a rapidly growing lymphoid tumor. Several allotypes present in the preinoculation sera were absent in postinoculation sera. When chickens heterozygous for allotypic loci were injected, usually only one of the two allotypes disappeared. The phenomenon was most pronounced for locus c specifities.

Allotypes in the Rabbit

Oudin (1956) produced six isoimmune sera in rabbits by injecting immune precipitates emulsified with paraffin oil adjuvant. Seven allotypes designated a, b, c, d, e, f, and g were found in rabbits using the same methods (Oudin, 1960a). The latter specificities appeared to be inherited (Oudin, 1960b).

Isoprecipitins produced by Dray and Young (1958, 1959) were directed against antigens which were apparently genetically determined. Tests on 500 normal sera obtained from several breeds of rabbits showed that the individual rabbits contained one or both of two gamma globulin antigenic specificities, RGG-I and RGG-II. Data on 335 offspring from 81 litters indicated that the latter allotypes were controlled

by a single pair of autosomal alleles, gamma^{I} and gamma^{II} (Dray and Young, 1960).

A study of the genetics of the allotypes A1, A2, A3, A4, and A5 was made by Dray et al. (1963). A1, A2, and A3 are determined by three autosomal alleles at a locus a, while A4 and A5 segregate as alleles at locus b. Two additional allotypes, P and T, were also identified. P is apparently controlled by a third locus, c, and is associated with the light immunoglobulin chains (Mage et al., 1968). No further studies have been conducted on allotype T.

Using precipitins obtained by injecting immune agglutinates, Dubiski et al. (1959) divided rabbit sera into two serum groups, D(a+) and D(a-). The D^{a} antigen had gamma globulin electrophoretic mobility, appeared to be inherited, and could be transmitted non-genetically from mother to fetus. Dubiski et al. (1961) described five isoantigens, A (D^{a}), B, C, D, and E, of which three were distinguished as genetic systems A, C, and BDE.

Several allotypes in rabbits have been identified more recently. Specificity As8 was detected using anti-allotype serum prepared by injecting Proteus vulgaris coated with antibody (Hamers et al., 1966). The allotype is controlled by a gene which seems to be linked with the a allotype locus. Another allotype, A10, also appears to be genetically linked to locus a. Allotypic specificity A9 is controlled by an

allele at the b locus (Dubiski and Muller, 1967). Mandy and Todd (1968) detected A11 using hemagglutnation-inhibition. The same authors detected allotypes A12 and A13 (Mandy and Todd, 1969). The antigens A11, A12, A13 were found to be controlled by a separate locus, independent of a and b, tentatively designated Ad. A fourteenth allotype, A14, has also demonstrated simple mendelian segregation (Dubiski, 1969). The gene controlling A14, Ae^{14}, is evidently closely linked with locus a. Finally, Conway et al. (1969a, 1969b) found that three alleles at locus f control the expression of five additional allotypes in three phenogroup combinations, f(1,5), f(2,4), and f(3,4).

Allotypic specificities are not restricted to the serum in rabbits. Thus, Feinstein (1963) found a colostrum component with electrophoretic mobility of a beta globulin which contained the allotypic specificities A4 and A5. Pernis et al. (1968) found that colostral IgA and serum IgG molecules contained the same heavy chain allotypic markers. A new gamma A immunoglobulin allotype in colostrum was discovered by Masuda et al. (1969). The specificity, c^1, was first detected by isoimmunization using a donor whose genotype was $a^2a^2\ b^4b^4$. The c^1 marker was present in rabbits with genotypes other than $a^2a^2\ b^4b^4$ also.

Lipoprotein allotypic determinants were reported by Albers and Dray (1969b). The authors identified four allo-

types of rabbit low-density lipoproteins, Lpq-1, Lpq-2, Lpq-3, and Lpq-4. The four determinants are inherited as phenogroups controlled by three codominant alleles, $q^{1,3}$, $q^{2,3}$, and $q^{2,4}$ (Albers and Dray, 1969a). In heterozygotes, only one of the two alleles contributes to the formation of the same molecule. Approximately 50 percent of the molecules had two specificities controlled by one allele and the other 50 percent had two specificities controlled by an alternate allele. For example, in a $q^{1,3}$ $q^{2,3}$ individual, half of the molecules had both q^1 and q^3; the other half had q^2 and q^3. None, however, had q^1 and q^2; hence, q^1 and q^2 are on different molecules (Albers and Dray, 1969a).

Immunochemical analysis has revealed that the allotypes controlled by the a locus (A1, A2, A3) are located on the Fd part of the heavy immunoglobulin chains (Dray et al., 1962; Stemke, 1964; Oudin, 1966; Kelus and Gell, 1967). The allotypes determined by alleles at locus b (A4, A5, A6, A9) are present on the light chains (Feinstein et al., 1963; Reisfield et al., 1965; Oudin, 1966; Dubiski and Muller, 1967; Appella et al., 1968). Determinants A4 and A5 are located on the antibody combining fragments I and II, obtained by papain digestion (Leskowitz, 1963). Allotype A7 is located on the light chains (Dray et al., 1963; Mage et al., 1968), while A8 and A10 are apparently on the heavy chains (Hammers et al., 1966; Hammers and Hammers-Casterman, 1967; Dubiski,

1969). Allotypes A11, A12, and A13 are carried by intact immunoglobulins, but evidently are not present on separated heavy and light chains (Mandy and Todd, 1968; Dubiski, 1969). The Fc portion of the heavy chain of IgG carries the A14 specificity (Dubiski, 1969).

The allotypes of the a locus in rabbits were originally found only on IgG molecules. However, Todd (1963) detected a locus allotypes on 19S molecules in serum as well as on 7S gamma globulin. More recently, the gamma G immunoglobulin heavy chain determinants have been found on gamma M immunoglobulin of serum (Stemke and Fischer, 1965; Todd and Inman, 1967) and gamma A of colostrum (Lichter, 1967). Pernis et al. (1968) reported similar results and concluded that there are no differences in the antigenic expression of the a locus allotypes present on the rabbit gamma and alpha chains. A major portion of IgA molecules carry locus a determinants (Kindt et al., 1968).

Light chain allotypes of the b locus have also been identified for the three major classes of immunoglobulins. The Ab antigens were found on IgG and IgM (Hoyer and Mage, 1967; Todd and Inman, 1967) and on IgA (Kindt et al., 1968). Todd and Inman (1967), working with both a and b locus determinants, indicated that nearly all of the antibodies against specificities on IgM molecules were capable of reacting with IgG, although not all antibodies against IgG

allotypes were capable of reacting with IgM.

Allotypes of antibodies involved in rabbit passive cutaneous anaphylaxis (PCA) reactions include both a and b locus determinants (Kindt and Todd, 1969). The PCA antibodies seem to belong to a new class of rabbit immunoglobulins. Thus, a and b locus allotypes have so far been detected on all identified classes of immunoglobulins in the rabbit.

Serum Isoantigens of the Mouse

An isoantigen in mouse gamma globulin was discovered by Kelus and Moor-Jankowski (1961). Individuals of one inbred strain were immunized against Proteus vulgaris. Proteus cells were then coated with anti-Proteus antibody and the complex was injected subcutaneously into a second strain twice per week. Tests in 1.5 percent agar gel plates revealed the allotype, gamma-B^A.

A second isoantigen in the mouse, Gg-2, is controlled by a single gene, Gg^2 (Wunderlich and Herzenberg, 1963). Two other allotypes, MuB1 and MuB2, have been identified (Cinader and Dubiski, 1963; Dubiski and Cinader, 1963). MuB1 is probably a component of hemolytic complement, C'5 (Kelus and Gell, 1967; Potter and Lieberman, 1967b). If so, it is not immunoglobulin. These studies were based on only a few inbred strains of mice. When additional donor-recip-

ient combinations were used, more elaborate systems of antigenic determinants were delineated. Thus, Lieberman and Dray (1964) detected five gamma globulin allotypes determined by the alleles Asa^1, Asa^2, Asa^3, Asa^4, and Asa^5. The Ig-1 locus has eight alleles (Ig-1a - Ig-1h) controlling isoantigens on the mouse 7S gamma-2a immunoglobulins (Herzenberg et al, 1965). Each antigen crossreacts with antisera against the other antigens in the group. Thus the antigens have similar determinants.

Isoprecipitins to a myeloma gamma A immunoglobulin in the mouse have detected allotypic determinants on the Fc portion of the heavy chain (Lieberman and Potter, 1966). Three allotypes were identified in 38 inbred strains. Of 758 mice tested for two determinants on the heavy chains of gamma G and gamma A immunoglobulin, 366 had neither specificity while the remainder showed both. No genetic recombinants were found; thus, the antigens seem to be controlled by closely linked loci.

Several alleles at each of at least four loci, Ig-1, Ig-2, Ig-3, and Ig-4, control allotypic determinants in the mouse (Herzenberg et al, 1967; Warner and Herzenberg, 1967). Of 200 F_2 progeny tested for linkage between Ig-4 and Ig-1, Ig-2, Ig-3, no recombinant types were found.

Antigenic determinants in the mouse have been detected on the Fc part of the heavy chains of gamma A, gamma F,

gamma G, and gamma H myeloma proteins, but none have been found on the light chains (Mishell and Fahey, 1964; Dray et al, 1965; Lieberman and Potter, 1966; Potter and Lieberman, 1967a). The gamma G heavy chain determinants (designated 1, 6, 7, and 8) were found to be widely distributed among 39 inbred strains examined by Potter and Lieberman (1967a, 1967b). Determinant G1 was present in four of the 39 strains, G6 in 19 strains, G7 in 22 strains, and G8 in eight strains. The gamma H heavy chain had specificities H9 and H11. Only three genetic combinations have been identified (H^{9}, $H^{9,11}$, H^{-}) among 38 inbred strains. The gamma A determinants, A12, A13, and A14, do not usually reach detectable levels in the serum until the mice are at least two months old (Potter and Lieberman, 1967b). Unique polymorphisms of the gamma F heavy chain have not, as yet, been identified. Thus far, five determinants (2, 3, 4, 5, and 10) have not been assigned to a specific chain (Potter and Lieberman, 1967b).

Serum Groups in Man

Grubb and Laurell (1956) showed that certain rheumatoid arthritic sera agglutinate group O Rh-positive red cells coated with selected incomplete anti-Rh antibodies. Human sera were classified by their ability to inhibit the rheumatoid agglutination using the phenotypic designations Gm(a+) and Gm(a-). The inhibitor was located in the gamma globulin

fraction of the serum. Also using a hemagglutination-inhibition test, Harboe (1959) detected another Gm factor, Gm^b. A serum group in a separate genetic system, Inv, was discovered by Ropartz et al. (1961). More recently, Martensson et al., (1966) reported Gm(s) and Gm(t).

Antisera capable of detecting two additional genetic factors (Gm(z) and Gm(y)) on the IgG1 class of the heavy chains of gamma G globulin were developed by Litwin and Kunkel (1966b). The antigenic determinant Gm(z) was found on all Gm(a+) myeloma proteins on the Fd piece of the heavy chain, while Gm(y) was located on the Fc fragment of myeloma proteins. The four genetic factors Gm(a), Gm(f), Gm(y), and Gm(z) were located on different fragments of the same myeloma protein. These factors segregated as a unit, suggesting close linkage or a single phenogroup.

More than 25 isoantigens of the Gm and Inv groups of human immunoglobulin G have been identified by passive hemagglutination (Ceppellini, 1967; Cohen and Milstein, 1967; Vyas and Fudenberg, 1969). In addition, Vyas and Fudenberg (1969) reported the first genetic marker for human immunoglobulin A. The determinant, Am(1), shows simple mendelian segregation and is independent of the Gm and Inv systems. Am(1) is located on the alpha chain of the IgG2 subclass.

The Gm factors have been found only on the heavy chains of the four types of IgG molecules (IgG1, IgG2, IgG3, IgG4). On the other hand, the Inv groups have been detected in IgG, IgA, and IgM, and on Bence-Jones proteins (Martensson, 1966; Cohen and Milstein, 1967; Terry et al., 1969). Inv factors are evidently restricted to the light chain. The distribution of Gm activity varies with different specificities. For example, Gm(a), Gm(x), Gm(y), Gm(b), and Gm(n) have been found on the Fc part of the heavy chain, whereas Gm(f) and Gm(z) have been found on the Fd fragment.

Specificities of the Gm system are transmitted genetically in certain fixed combinations, the combination being different for different races (Martensson, 1966; Oudin, 1966; Cohen and Milstein, 1967). Each set acts as a unit of inheritance in family studies. No genetic recombinations have been observed. Some of the known genetic combinations or phenogroups have been summarized by Ceppellini (1967). Most individuals seem to carry genes for both Gm(a) and Gm(b). Whether or not these genes are expressed in the phenotype, however, seems to depend on regulatory genes which control the cistrons coding for Gm(a) and Gm(b) (Lobb et al., 1967). Apparently each set of specificities is determined by a cluster of sites on one chromosome, each site being a unit of inheritance (gene), and sets of specificities make up a series of alleles (Cohen and Milstein, 1967) However,

since the Gm determinants are confined to the heavy chains of the four major subclasses of IgG (IgG1, etc.), some investigators have suggested there may be four closely linked loci, each directing the synthesis of one type of chain (Martensson, 1966; Cohen and Milstein, 1967). For example, according to this theory, the IgG1 locus controls the synthesis of Gm(a), Gm(f), Gm(y), and Gm(z). On the other hand, Kaarslo (1969) attributes control of Gm specificities to three cistrons, IgG2, IgG3, and IgG1, in the order they appear on the chromosome.

Human immunoglobulin factors have been studied immunochemically. The relationship between the Inv phenotypes and the amino acids at position 191 in kappa light chains was studied by Terry et al. (1969). In seven Inv(-1,3) homozygotes, the kappa chains had valine at position 191, while three Inv(1,3) heterozygotes had some chains with valine and some with leucine. Genes coding for Inv(1) and Inv(3) antigens apparently are expressed equally in heterozygotes, since about equal quantities of these antigens were recovered from the heterozygotes. Thus, the valine-leucine interchange is evidently coded by two alleles at one locus.

The rheumatoid factors, used to test for Gm variants, are antibodies directed against determinants in the Fc part of the IgG heavy chains. A recent study by Natvig and

Turner (1970) indicated that these factors are directed against at least two areas in the Fc fragment. One is the Gm(a) site; the other is the N-terminal half of the Fc region where Gm(g) and Gm(b) are located.

Allotypes in Other Animals

Several other species of animals have shown isoantigenic specificities of serum. Two mg. of pooled rat serum (Wistar BB strain) in Freund's complete adjuvant injected intraperitoneally into a second strain produced an isoprecipitin. The latter was used to detect the first serum isoantigen, Ral, found in rats (Barabas and Kelus, 1967). Wistar (1969) reported an allotype in rats, RI-1, present on the light chains of IgG and IgM molecules. Two gamma globulin antigens identified in pigs using a hemagglutination-inhibition test (Rasmusen, 1965) have shown two codominant alleles, Gl^a and Gl^b, determining three phenotypes, Gl(a+b-), Gl(a+b+), and Gl(a-b+). Heteroimmunizations, followed by absorptions, were used by Iannelli *et al.*, (1966) to produce isoantibodies in cattle. Some normal cattle sera were precipitated by the antisera in gel diffusion and some were not. Rapacz *et al.* (1968) described an allotypic specificity, Mcl, in cattle. The antigen was located on the IgM molecule

and was controlled by an autosomal gene. More recently, Dykstra[1] also found serum isoprecipitins in cattle. Allotypic specificities have also been reported in guinea pigs, (Benacerraf and Gell, 1961), baboons (Kelus and Moor-Jankowski, 1962), and ducks (Kaminski and Ligouzat, 1964).

Production of Allotype Isoprecipitins

In the rabbit, anti-allotype antiserum is made by injecting globulin from a donor carrying a particular allotype antigen into a recipient lacking that antigen (Mage and Dray, 1965; Sell and Gell, 1965; Kelus and Gell, 1967). Isoprecipitins in the mouse and the rat are produced by immunization of individuals of one inbred strain with immunoglobulin from individuals of another inbred strain (Dray et al., 1965; Herzenberg et al., 1965; Barabas and Kelus, 1967; Warner and Herzenberg, 1967; Wistar, 1969).

Several variations in techniques of producing anti-allotype reagents have been used. Dubiski et al. (1959, 1961) immunized rabbits against several species of bacteria, including Proteus vulgaris X19, Salmonella typhimurium, and Escherichia coli. The resulting anti-bacterial serum was mixed with a suspension of the respective bacterial cells.

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[1]Dykstra, Dennis. Department of Veterinary Microbiology, Iowa State University of Science and Technology, Ames, Iowa. Private communication. 1970.

The mixture was incubated for an hour at 37°C., washed three times in saline, resuspended in saline, and injected intravenously twice weekly for five to seven weeks. Each recipient received agglutinate from only one donor. Sera were tested using Ouchterlony's gel diffusion technique. Similar procedures using different carrier antigens have been employed in chickens. Proteus vulgaris was used as the carrier antigen by Skalba (1964a, 1966), while David (1966) made subcutaneous injections of the complex: Brucella abortus - anti-B. abortus emulsified in Freund's incomplete adjuvant.

Coliform bacteria, such as Proteus vulgaris, seem to be excellent carrier antigens in rabbits, producing strong antisera against allotypes. This advantage seems to be due to the strong adjuvant effect of the endotoxin produced by the bacteria (Kelus and Gell, 1967).

Three variations in procedure were tested by Leskowitz (1963) to produce allotype reagents in rabbits. In procedure 1, 11 intravenous injections of one mg. of alum-precipitated gamma globulin, carrying allotypes A4 and A5, were made into recipients carrying either A4 or A5 but not both. In procedure 2, the allotype antigens were emulsified in complete adjuvant. About 0.4 ml. of emulsion was then injected into four toe pads and into two intramuscular sites. A second intramuscular injection was given two weeks later.

In procedure 3, bovine serum albumin - rabbit anti-bovine serum albumin was emulsified in adjuvant and injected into appropriate recipients. All three methods proved to be about equally effective in producing isoprecipitins. Booster injections were ineffective in increasing antibody titer for the adjuvant injected animals. In one case, the antibody titer declined to a nearly undetectable level following a booster injection.

Heteroimmunizations were used by Bornstein and Oudin (1964) to make antibodies against allotypes in rabbits and by Litwin and Kunkel (1966a) to produce antisera against serum isoantigens in humans. The former used chickens and goats for recipients while the latter injected rabbits. Appropriate absorptions were made to remove species-specific antibodies.

Whole normal serum has been successfully used to make isoprecipitins in the rabbit (Dray and Young, 1958; 1959), in the mouse (Dubiski and Cinader, 1963), and in the chicken (David, 1966). The serum was injected with complete adjuvant and the resulting isoprecipitins were then used to detect allotypic determinants.

Cellular Location of Allotypes

Data on the cellular localization of allotype production is somewhat conflicting but the one cell - one

allotype theory is favored. Evidence that immunoglobulin molecules which carry two allelic specificities were not synthesized by the same cells was reported by Reider and Oudin (1963). However, Colberg and Dray (1964), using fluorescent antibody techniques to detect allotypic specificities A4 and A5 (b locus), found that less than one percent of the cells contained only one allotype. They concluded that the cells were capable of producing both allelic products. Pernis et al. (1965), on the other hand, found two populations of plasma cells, one which contained one allotype and a second containing the alternative allotype. Similarly, allotypic markers Aa1 and Aa2 have been found in different cells of a^1a^2 heterozygous rabbits (Cebra et al., 1966). In human immunoglobulin studies, fluorescent antibodies to the allelic characters Gm(a) and Gm(b) were found in different plasma cells in the red pulp of the spleen and the medullary cords of the lymph nodes of Gm(a+b+) individuals (Curtain and Baumgarten, 1966). But in the germinal centers of the lymph nodes and white pulp of the spleen, both serum groups seemed to be present in the same cells.

Lymphocytes carry allotypic determinants (Coombs et al., 1970). Antisera directed against these allotypes stimulate the lymphocytes to transform into immature "blast" cells and to undergo division in vitro. Rabbit antisera to allo-

type Ab4 stimulated up to 77 percent blast transformation of b^4b^4 homozygous lymphocytes *in vitro* and a maximum of 39 percent transformation of b^4b^5 heterozygous lymphocytes (Sell, 1968a). Thus, the lymphocytes of an allotypically heterozygous rabbit may be selected to produce only one of two determinants controlled by the *b* locus. In an additional study by Sell (1968b), "the number of lymphocytes transformed *in vitro* by anti-allotype sera in cultures from allotypically suppressed rabbits was significantly less than that induced in cultures from normal rabbits." This was compensated by increased transformation induced by antisera against the unsuppressed allotype. Thus, the control of allotypic expression may be the same for lymphocytes as for plasma cells.

Other Allotype Studies

The contribution of allotype alleles b^4 and b^5 was studied by Dray and Nisonoff (1963). In homozygotes and heterozygotes 80 to 90 percent of the gamma globulin molecules contained A4 or A5, while 10 to 20 percent had neither. In heterozygotes, 64 percent of the molecules had A4 and 27 percent had A5. Colostral IgA from rabbits of different genotypes was analyzed individually for the number of molecules carrying locus *a*, *b*, and *f* specificities (Lichter *et al.*, 1970). The percentage of molecules bearing

allotypic specificities varied from 68 to 104 for locus a, 82 to 98 for locus b, and 62 to 107 for locus f. The levels of allotypes in serum is relative to the number of plasma cells producing the allotypes (Lummus et al., 1967).

Antibodies were made against two hapten determinants in rabbits (Lark et al., 1965). Every purified antibody contained the same allotypes present in the serum from which the antibodies were isolated. However, the quantity of certain allotypes for the haptens differed from that in the whole serum. In four a^1a^3 heterozygous rabbits, the quantity of Aa1 molecules was consistently less, and Aa3 molecules more, in antibodies directed against DNP than in the total gamma G immunoglobulin from which they came. Similarly, anti-SSS III antibodies usually occurred as Ab4 rather than Ab5 molecules (Catty et al., 1969). Ab-negative molecules contributed significantly to the antibody response. These results suggest that the antibodies were formed selectively from certain allotypes.

The phage-neutralizing activity of rabbit antisera carrying a locus specificities can be partially inhibited by anti-allotype sera (Stemke, 1969). Inhibition was greater than 90 percent in some cases, but some neutralizing activity remained. Thus, allotypic heterogeneity was also shown by rabbit antibodies capable of neutralizing phage.

Allotypic expression can be suppressed by immunizing a

mother rabbit against a paternal allotype. For example, if an a^3a^3 b^4b^4 doe is injected with globulin from an a^1a^1 b^4b^4 donor, anti-Aal antibodies are produced. If the doe is mated to an a^1a^1 b^5b^5 male, the a^1a^3 b^4b^5 offspring will have a very low level of Aal allotype in their sera (Mage et al., 1967). The altered phenotypic expression of a locus allotypes may persist for as long as 23 months. Suppression of allotypes determined by the b locus alleles persists for at least five months (Dray, 1962). In addition, a decrease in the quantity of one allotype was balanced by a compensatory increase of another allelic allotype. David and Todd (1969) produced in utero allotypic suppression. Embryos were transplanted into does producing antibody against an allotype present in the homozygous state in the embryo.

Gell and Kelus (1966) reported a lack of tolerance to allotypes in rabbits. Even though maternal immunoglobulins sometimes contained allotype determinants not present in the offspring's genotype, neither tolerance nor sensitization to these determinants were observed. In humans, sensitization has been observed. Anti-Gm(a) antibodies were found in Gm(a-) children from Gm(a+) mothers (Luczkiewicz-Mulczyhowa, 1968).

Multiple serum samples from a human (Gm(a-) female mated to a Gm(a+) male were obtained before, during and after each of four normal pregnancies (Fudenberg and Fudenberg, 1964). An agglutinator for Gm(a-) first appeared in the

mother's serum during the third pregnancy. The genotype of the newborn (male) was Gm(a+), since his serum contained small amounts of Gm(a+) gamma globulin, in addition to maternal Gm(a-) globulin. Based on the same principle, post transfusion shock has been produced in rabbits (Popielski et al, 1967). Thirteen of 21 rabbits immunized against gamma globulin produced anti-allotype antibodies. When the heterologous allotypes were introduced again later, the isoprecipitins against the allotypes apparently caused active post transfusion shock.

MATERIALS AND METHODS

Genetic Stocks

The breeds and crosses used in this project were:

W: A heavy-breed line developed from a cross between a Barred Plymouth Rock and a Rhode Island Red (Briggs, 1970).

GW,WG: Reciprocal crosses between W and a White Leghorn line (G) selected for low body weight and high egg weight (Casey, 1970).

S-lines: White Leghorn lines S1 and S2 segregating for B locus blood group alleles (Rishell, 1968).

DwX: Dwarf crosses described in Part II of this thesis.

I-9,I-19,I-GH,I-HN: White Leghorn inbred lines with approximately 54, 58, 70, and 85 percent inbreeding (Marangu, 1970).

Allotypes

The subpopulations studied for allotypic segregation were (David et al., 1969):

Subpopulation	Known allotypic system	Antigens	Alleles
A-a	Aa	Aa1,Aa2	Aa^1,Aa^2
A-b	Ab	Ab1,Ab2	Ab^1,Ab^2
A-c	Ac	Ac1,Ac2,Ac3,Ac4	Ac^1,Ac^2,Ac^3,Ac^4

The A designation of "allotype" adopted by Dray et al. (1962) was used when referring to the subpopulation but omitted with reference to the genetic system, antigen, and allele for the remainder of this thesis. The lower case letter designating the allotypic system followed by the number indicating the allelic specificity was retained.

Breeding generations

P_0: Parents of the initial crosses of the 1969 subpopulations.

F_1: Offspring of P_0.

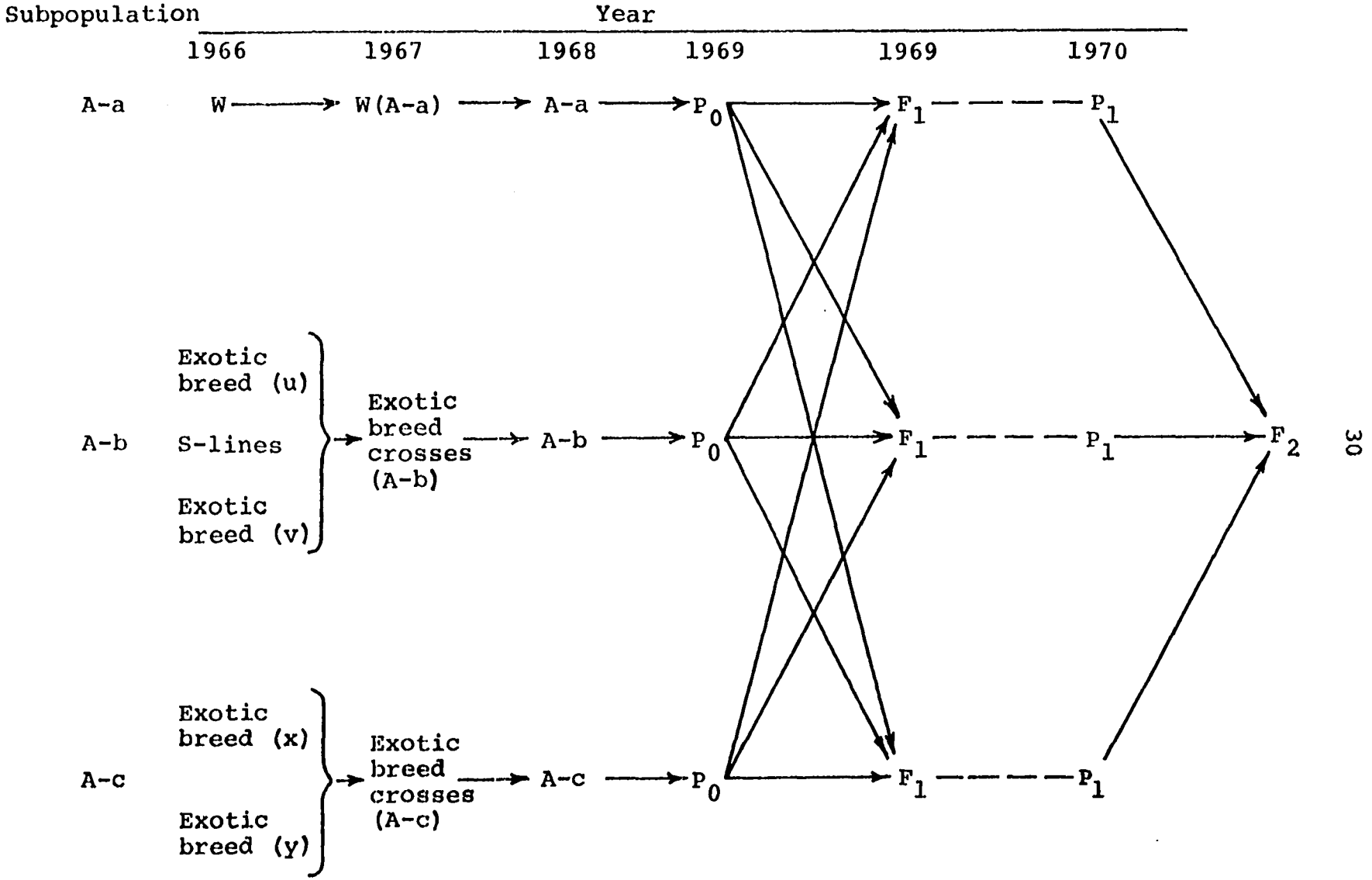
P_1: F_1 offspring used for the 1970 matings.

F_2: Offspring of P_1 matings.

Characterization of Populations

Most of the populations studied were chickens of diverse origin that David (1966) used for his original work on allotypes. The 1967 breeders were divided into three subpopulations, A-a, A-b, and A-c, corresponding to the main allotypic system found in the subpopulation. For example, if a bird carried a known b system specificity, but the a and c specificities were undetermined, it was assigned to the subpopulation A-b. The origins of subpopulations A-a, A-b, and A-c are shown in Figure 1. In 1967 each subpopulation was allotyped only for the known allotypes and not for those in the other subpopulations. In 1968, chickens from A-a, A-b,

Figure 1. Path diagram showing the formation of segregating populations with known allotypes in the F_2. The exotic breeds included Houdan, Speckled Sussex, Silver Laced Wyandotte, Red Leghorn, Buff Orpington, Buff Laced Polish, Dark Cornish, White Langshan, Buttercup, Blue Andalusian, Silver Speckled Hamburg, Silver Leghorn, Araucana, and Red Cap (David et al, 1969).


Subpopulation
Year
1966
1967
1968
1969
1969
1970
A-a
W
W(A-a)
A-a
P_0
F_1
P_1
A-b
Exotic breed (u)
S-lines
Exotic breed (v)
Exotic breed crosses (A-b)
A-b
P_0
F_1
P_1
F_2
A-c
Exotic breed (x)
Exotic breed (y)
Exotic breed crosses (A-c)
A-c
P_0
F_1
P_1

and a few from A-c were typed for the eight genetically identified allotypes. From these, the most complete genotypes were selected as breeders in an attempt to produce a single population segregating for known alleles only (P_0; Appendix, Table 69). The typing results for c2 were incomplete due to lack of antisera; hence, this antigen was disregarded. Those individuals already found to be heterozygous for known alleles at one or more loci were mated to continue the segregation. Full- or half-sib matings were avoided. Most of the matings were crosses between subpopulations with only a few within subpopulations.

The F_1 offspring from the matings shown in Appendix Table 69 were allotyped for the known antigens except c2. Matings in generation P_1 (Appendix, Table 70) were based on the same criteria as for the P_0 generation. All F_2 offspring were typed for a1, a2, b1, c1, c3, and c4. About three-fourths were typed for b2; none were typed for c2.

Allotyping Tests

Gel formula

Tests for allotypes were made by Ouchterlony's double diffusion in agar gel method. The gel composition was:

1 gm. Noble agar[1]

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[1]Difco Laboratories, Detroit, Michigan.

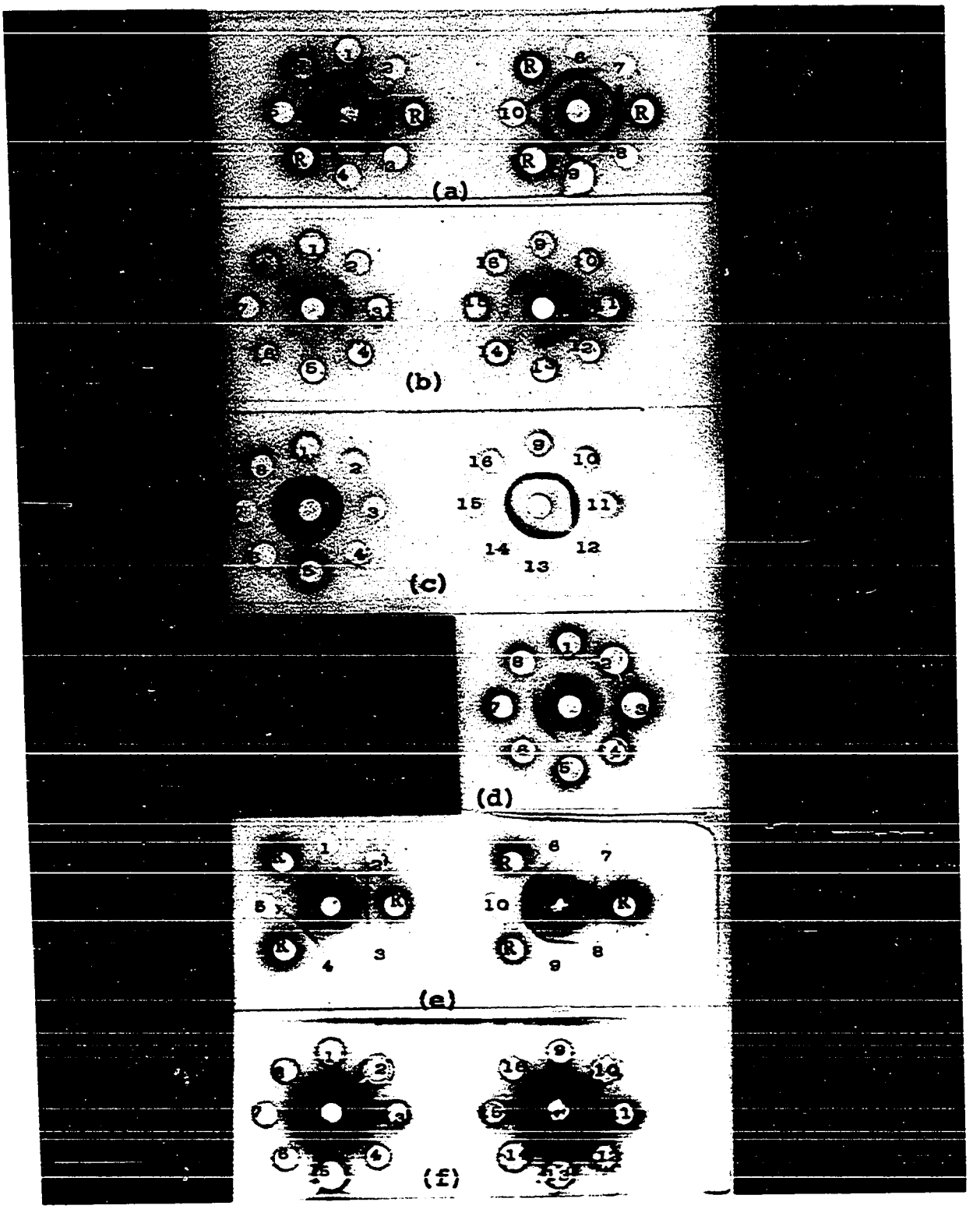
8 (9) gm.	Sodium chloride
35 ml.	Sodium phosphate dibasic (Na_2HPO_4), 0.15M
15 ml.	Potassium phosphate (KH_2PO_4), 0.15M
10 ml.	1:1000 aqueous merthiolate
50 ml.	Distilled water

The salt concentration was eight percent in the early tests but nine percent in later tests; the higher salt concentration seemingly gave slightly stronger reactions for at least one anti-allotype reagent. The above mixture was boiled for five minutes in a waterbath and the hot agar was pipetted onto clean microscope slides. After cooling to room temperature, the gels were stored in a refrigerator until used.

Testing schemes

Using a die, two parallel circles of eight wells each plus center wells were cut in the gels. Different test patterns were chosen for the various antisera (Figure 2). Allotypes a1 and c3 were tested with antiserum GH-745 in the pattern shown in Figure 2a, using a reference serum specific for c3. The allotypes a2, b1, and b2 were tested without reference sera (Figures 2b, 2c, and 2d). The a2 and b1 antisera (SSH-5912 and BLP-5837) were monospecific. The anti-b2 reagent (H-5968) had two specificities, Ax2 (unknown allotypic determinant x2) and b2. The b2 precipitation line formed near the antibody well (center), while the Ax2 line

Figure 2. Testing sera for allotypic specificities: The anti-allotype serum was placed in the center well and the unknown test sera were put in the peripheral wells. Test sera were typed for: (a) a1 and c3; the reference serum (R) was specific for c3. Test sera 2, 4, 7, 9, and 10 contained a1, while samples 1, 3, 5, 6, and 8 had both a1 and c3. (b) a2; test sera 1, 2, 3, 5, 6, 8, 10, 12, 13, and 16 contained a2. (c) b1; all test sera except 12 contained b1. (d) b2; all test samples except 8 had b2. (e) c1; test sera were typed alongside a known c1 reference serum (R); samples 9 and 10 had c1. (f) c4; test samples 1, 3 to 9, 11, 14, and 15 contained c4.


(a)
(b)
(c)
(d)
(e)
(f)

appeared nearer the antigen (peripheral) wells. Hence, a reference serum was not necessary. Figures 2e and 2f show the test patterns for c1 and c4. No tests were made for c2.

Staining technique

Test slides were stained with amido black 10B. Three solutions were necessary: an eight percent sodium chloride solution, a rinse solution containing 10 parts acetic acid, 45 parts methanol, and 45 parts distilled water, and a staining solution made up of 1500 ml. of rinse solution and nine grams of amido black 10B stain. Part of the treatment of gel slides included soaking in eight percent salt for eight hours followed by rinsing in distilled water for 45 minutes. After placing strips of filter paper over the gels, they were set aside to dry. Finally, the slides were placed in the staining solution for 10 minutes and passed through five rinses of 10 minutes duration each.

Because the above procedure produced non-specific staining, some of the results were inconsistent. However, the problem was nearly eliminated when the slides were given a slightly different treatment. First, they were rinsed in distilled water both before and after being dried, and then they were immersed in the staining solution for only one minute rather than 10 minutes. Finally, they were passed through five rinses as before but were kept in the last rinse for two to three hours rather than only 10 minutes.

Production of Allotype Antisera

Basic procedure

The basic procedure for making allotype antisera (Figure 3 and Table 1) was essentially the same as described by David (1966). The steps were:

(1) Chickens with known allotypic specificities, from subpopulations A-a, A-b, and A-c, were chosen as donors.

(2) The recipients, each with known allotypic specificities, were close relatives of donors. Recipients differed from donors by one known allotype; for example, if a b1 antiserum was desired, the donor might be a b^1b^2 heterozygote and the recipient a b^2b^2 homozygote.

(3) Prospective donors were injected intravenously three times weekly for three weeks with one ml. of Brucella abortus cells (washed three times in saline). One week after the final injection, blood, from which the anti-Brucella serum was harvested, was collected from the wing vein.

(4) One ml. of B. abortus (low dilution)[1] per recipient

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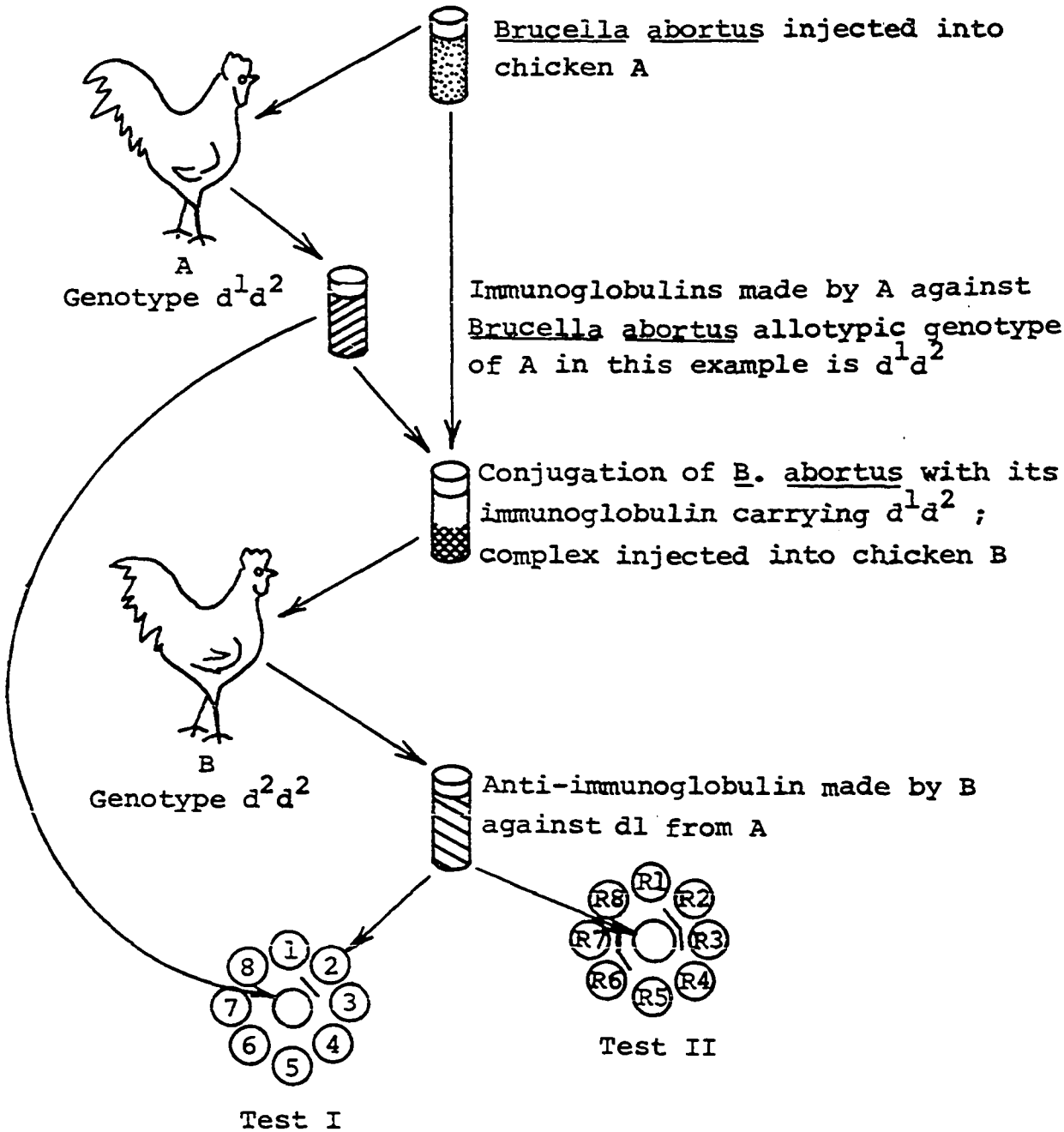
[1]A 4.5 percent concentration of Brucella abortus tube antigen, serial number T-110, supplied by the Animal Health Division of the Agricultural Research Service, National Animal Disease Laboratory, Ames, Iowa.

Figure 3. Production of antiserum against a hypothetical allotype d1 (modified from Law, G. R. J., Hy-Line Poultry Farms, Johnston, Iowa and David, 1966). Test I: Serum in center well from chicken A carrying d1 reacts with anti-d1 antiserum (made in chicken B) in peripheral well number 2. Test II: Antiserum against d1 in center well reacts with normal reference sera, containing d1, in peripheral wells 2, 3, 6, and 7.


Brucella abortus injected into chicken A
A
Genotype d^1d^2
Immunoglobulins made by A against Brucella abortus allotypic genotype of A in this example is d^1d^2
Conjugation of B. abortus with its immunoglobulin carrying d^1d^2 ; complex injected into chicken B
B
Genotype d^2d^2
Anti-immunoglobulin made by B against dl from A
1
2
3
4
5
6
7
8
Test I
R1
R2
R3
R4
R5
R6
R7
R8
Test II

was centrifuged at 3300 rpm for thirty minutes. The supernatant was then discarded.

(5) One ml. of donor anti-Brucella antiserum per recipient was added to the packed Brucella cells.

(6) After thoroughly mixing the B. abortus plus antiserum, the agglutinated cells were incubated in a 37°C. waterbath for one hour.

(7) The mixture was centrifuged and the antigen-antibody complex was washed twice in saline.

(8) For each recipient, 0.5 ml. of the complex plus saline was emulsified in 0.5 ml. of Freund's incomplete adjuvant.[1]

(9) One ml. of the above was injected into a chicken at four or five different sites subcutaneously at bi-weekly intervals. Recipients which produced precipitins were given booster injections at monthly intervals; otherwise, immunization was terminated at five injections.

(10) Recipients were bled seven days after injection beginning with injection 2.

(11) Recipient sera were tested by Ouchterlony's double diffusion in gel method (Figure 4).

(12) Slides from (11) were stained with amido black 10B

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[1]Difco Laboratories, Detroit, Michigan.

by the method already described.

Modifications Because the basic procedure seemed not to be effective in producing antisera, several modifications, listed below according to the step number of the basic procedure, were tried (Table 1).

(2a) Family relationship between donor and recipient was ignored; also, some recipients differed from donors by more than one known allotypic determinant.

(2b) Recipients were chosen from different populations with unknown allotypic specificities rather than from the allotype subpopulations.

(3a) Some recipients in early injection schedules were used later as donors since their sera carried both known allotypic specificities and anti-B. abortus antibodies. Hence, they were immunized against the carrier antigen via the subcutaneous route for up to 12 weeks rather than by the intravenous route for three weeks.

(4a) Two to five ml. of Brucella abortus per recipient, rather than one ml., were centrifuged.

(9a) Injections were not always two weeks apart but were 10 days to 15 weeks apart.

Precipitin tests The tests (Ouchterlony's gel diffusion) were set up in two ways. In the first, the donor

Table 1. Summary of steps in basic and modified procedures followed for the development of allotype antisera

Injections	Steps[a] 1	2	3	4	5	6	7	8	9	10	11	12
1968	(unmodified basic procedure used)											
1969												
Series I		2a		4a								
II		2a 2b	3a	4a								
III		2a 2b	3a									
IV		2a 2b	3a	4a					9a			
V		2a 2b	3a	4a					9a			

[a]The basic procedure was used unless otherwise specified.

serum was placed in the center well and the recipient sera in the peripheral wells (Figure 4a). Later, the test pattern shown in Figure 4b proved to be more satisfactory because the peripheral wells were closer together and more distant from the center well. The donor serum was placed in the center well and three peripheral wells. The recipient sera were then placed in the remaining peripheral wells. New anti-allotype antisera and standard antisera were tested against donor sera in alternating peripheral wells as shown in Figure 5. New antisera were also tested against known allotype reference sera (Figure 6).

The numbers of donors and recipients used in different immunization schedules are summarized in Table 2. Complete descriptions of donors and recipients are given in Appendix Tables 71 through 76.

Immunizations in 1968

The basic procedure was followed without modifications in 1968. For the A-b subpopulation, donors and recipients usually consisted of full-sib or half-sib groups.

Immunizations in 1969

Five series of injections were made in 1969. The procedures, including modifications (Table 1), are listed below by series.

Series I injections - The basic procedure was followed

Figure 4. Testing recipient sera against donor serum (D): Recipient sera were placed in consecutively numbered peripheral wells (a) or they were alternated with the donor serum (b). Recipients 1, 2, and 3 contained antibody against determinants in the donor serum.

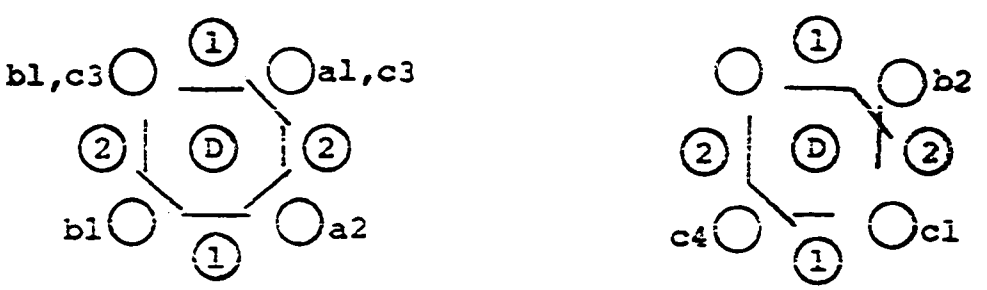


Figure 5. Testing new antisera (1 and 2) against the donor serum (D), alongside known standard antisera (for example, a1, c3 was the standard for a1 and c3). No c2 standard was available. Recipient 1 evidently carried a precipitin against b2, while recipient 2 had antibody against c4. Additional tests with known allotype reference sera would be needed to verify the specificities.

Figure 6. Testing a new antiserum (r) against known allotype normal reference sera (R). Donor serum (D) was the control. The new antiserum contained antibody against an antigen in R1, R3, and R4.

Table 2. Numbers of donors and recipients used in different immunization schedules

Year	Injection series	Number of donors	Number of recipients
1968	--	10	45
1969	I	9	29
	II	8	26
	III	8	33
	IV	8	39
	V	12	61

except that family relationships between donors and recipients were ignored (modification 2a) and the amount of antigen centrifuged for mixing with anti-Brucella serum was increased, beginning with the second injection, by one ml. for each injection, up to five ml. for the fifth injection (modification 4a). In addition, a complex of Brucella + anti-Brucella in saline was injected intravenously one month after the fifth injection. The recipients were then bled five and 10 days later and the sera tested by gel diffusion.

Series II injections - The same procedure was followed as for series I, with additional modifications. Four of the donors were immunized against B. abortus by subcutaneous, rather than by intravenous, injections (modification 3a). Donor serum was injected into recipients from: (1) the same

allotype subpopulation as that of the donor, (2) a different allotype subpopulation from that of the donor, and (3) new populations (modification 2b).

Series III injections - The procedure was similar to that for series II. The recipients were mostly S1 chickens (modifications 2a and 2b). All donors were formerly recipients in the 1968 injections (modification 3a). Also, the series III birds were given a "rest" period of 15 weeks after injection 5. They were then treated as described in the basic procedure without modification.

Series IV injections - All modifications were used in this series of injections. Most of the recipients were Leghorn x heavy crossbreds GW and WG, with a few S-line and W birds in addition (modifications 2a and 2b). All donors were formerly recipients in the 1968 injections (modification 3a). The amount of Brucella centrifuged for mixing with anti-Brucella serum was increased to five ml. beginning with the first injection (modification 4a). Also, the first two injections were two weeks apart as usual but injections 2 and 3 as well as 3 and 4, were only 10 days apart (modification 4a). Except for one donor, the fifth injection was omitted.

Series V injections - Sixty-three recipients of the following types were used: (a) chickens from the allotype subpopulations which had not previously been injected, (b) chickens from the allotype subpopulations which had been

recipients in a previous injection series for a different donor, and (3) inbreds (modifications 2a and 2b). Full- or half-sibs of the donor were used exclusively in (a) and (b). For (b), it was possible that an antigen in the donor serum had a determinant similar to determinants in the previous donor serum. Thus, an antibody of undetectable titer might have been produced originally. If so, introduction of a related antigen might have acted as a booster injection. Type (c) recipients were inbred Leghorn lines 9, 19, and GH.

Six of the 12 donors were previously recipients in series I and II (modification 3a). Modification 4a was applied for the first four injections (using four to five ml. of Brucella cells) with one exception: the anti-Brucella titer of one donor serum was too low for the high concentration of antigen. Thus, the modification was not applied to this donor. Also, the basic procedure step (4) was used without modification for injection 5. The first three injections were each two weeks apart as usual. Injections 3 and 4, however, were four weeks apart, while injections 4 and 5 were five weeks apart (modification 9a).

A summary of the procedures used for the different series is given in Table 1.

Miscellaneous injections

Three additional approaches to making anti-allotype antisera were tried. First, two turkeys were injected, each with a different donor serum once on each of two successive weeks. They were then bled seven days after the second injection. Otherwise, the same procedure as for chickens was followed without modification. Antiserum production was also attempted by first precipitating the donor serum proteins with 20 percent sulfosalicylic acid. The aggregated proteins were washed in saline and resuspended to a 25 percent concentration. For each of eight recipients of inbred line HN, 0.5 ml. of protein was mixed with 0.5 ml. of Freund's complete adjuvant and then injected subcutaneously into four sites. Three injections were spaced two weeks apart and the birds were bled one week after the second and third injections. The sera were tested against the donor serum by Ouchterlony's double diffusion in gel method.

Finally, a technique using egg yolks was tried for making allotype antisera. In a yolk antibody - yolk allotype study in our laboratory (unpublished data), the b1 allotype was detected in the yolk while a1, a2, and c3 were not. In addition, anti-Brucella abortus antibodies were found in the yolk of immunized hens. Hence, it seemed that the b1 antigen was present in isolated form and that a mono-specific antiserum might be made using yolk globulin, with B. abortus as the carrier antigen. Anti-Brucella antibody was extracted

from the yolks by first mixing whole yolk 1:5 in saline, followed by centrifugation.[1] A layer of lipid material was then removed with a pipette and the aqueous layer, containing anti-B. abortus, was substituted for the serum in the basic procedure already described. Yolk extract from two donor hens were injected into each of three recipients (inbred line HN). The latter were then bled one week after a single injection; otherwise, the basic procedure was followed without modification.

[1]Method suggested by Dr. M. L. Frey, Veterinary Medical Research Institute, Ames, Iowa.

RESULTS

Allotype Populations

The typing results for the F_1 generation are given in Appendix Table 77. Some of the phenotypes for the F_1 are lacking because errors in classification were found after the initial tests. Only retested progeny or those from sires or dams with undetected errors are listed. Unfortunately several males with questionable phenotypes were discarded in the interest of economy before they could be retested.

The F_2 results are given in Appendix Table 78. No offspring were tested for C2 and offspring numbers 14626 through 14675 were not typed for b2.

From the results we suspected that some of the genotypic designations for the allotypes were in error. In an attempt to detect these, observed ratios were compared to expected ratios. The F_1 progeny which were typed twice, plus all F_2 chickens, were included in the comparisons. The mating types and segregations for a^1 and a^2 are listed in Table 3. The chi square test supports the hypothesis that a^1 and a^2 are codominant alleles although the fit was not especially good for two of the mating types. Because the numbers of offspring were small for any one mating, sampling variation would be large.

Table 4 gives the segregation of the a^1 allele ignoring a^2. The results show that a^1 segregates according to simple mendelian expectation. Similarly, in Table 5, a^2 shows

Table 3. Segregation of a^1 and a^2

Deduced parent genotype[a] sire	dam	Number of: matings	progeny		Phenotype of progeny[a] a1	a1a2	a2	—	Probability of larger χ^2 value
a^1a^1	a^1a^2	10	77	Observed	39	38	0	0	0.90
a^1a^2	a^1a^1			Expected	38.5	38.5	0	0	
a^1a^1	$a^2/-$	4	19	Observed	7	12	0	0	0.25
$a^2/-$	a^1a^1			Expected	9.5	0.5	0	0	
$a^1/-$	a^1a^2	4	16	Observed	8	3	5	0	0.50-0.75
a^1a^2	$a^1/-$			Expected	8	4	4	0	
$a^1/-$	$a^2/-$	5	33	Observed	12	10	7	4	0.01-0.025
$a^2/-$	$a^1/-$			Expected	8.25	8.25	8.25	8.25	
a^1a^2	$a^2/-$	2	20	Observed	5	8	7	0	0.25-0.50
$a^2/-$	a^1a^2			Expected	5	5	10	00	
a^1a^2	a^1a^2	1	9	Observed	1	6	2	0	0.10-0.25
				Expected	2.25	4.5	2.25	0	
a^1a^2	a^2a^2	5	37	Observed	0	15	22	0	0.25
a^2a^2	a^1a^2			Expected	0	18.5	18.5	0	

[a]The dash means not allotypes a1 or a2 or their alleles.

Table 4. Segregation of a^1 ignoring a^2

Deduced parent genotype[a] sire	dam	Number of: matings	progeny		Phenotype of progeny[a] a1	—	Probability of larger χ^2 value
$a^1/-$	$a^1/-$	5	25	Observed	18	7	0.50-
				Expected	18.75	6.25	0.75
$a^1/-$	$-/-$	12	90	Observed	50	40	0.25-
$-/-$	$a^1/-$			Expected	45	45	0.50
a^1a^1	$a^1/-$ or $-/-$	18	133	Observed	113	0	
				Expected	113	0	

[a]The dash means not allotype a1 or its allele.

Table 5. Segregation of a^2 ignoring a^1

Deduced parent genotype[a] sire	dam	Number of: matings	progeny		Phenotype of progeny[a] a2	—	Probability of larger χ^2 value
$a^2/-$	x $a^2/-$	3	29	Observed	23	6	0.50-
				Expected	21.75	7.25	0.75
$a^2/-$	x $-/-$	23	145	Observed	75	70	0.50-
				Expected	72.5	72.5	0.75
$-/-$	x $a^2/-$						
a^2a^2	x $a^2/-$	5	37	Observed	37	0	
				Expected	37	0	
$a^2/-$	x a^2a^2						

[a]The dash means not allotype a2 or its allele.

simple mendelian segregation.

The alleles b^1 and b^2 were probably segregating as codominants although not all of the tests consistently supported this hypothesis (Table 6). The "excess" of the b1b2 phenotype in the first two matings in Table 6 might be due to errors in phenotyping for b2. Reactions found using the b2 antiserum were weak and the precipitates formed closely adjacent to the antibody well; hence, the b2 tests were sometimes difficult to interpret. The b1 reactions were easy to read because they were strong and clear. Also, because the chickens were typed only once for b2 due to lack of antiserum, consistency of the b2 reactions was unknown. Reactions of b1 with its antiserum were completely repeatable between tests.

Segregation of b^1 ignoring b^2, and of b^2 ignoring b^1, is given in Tables 7 and 8. The distribution of the allotypes produced by both b^1 and b^2 seems to follow simple mendelian expectation.

Contrary to the conclusions of David et al. (1969), allotypes c1, c3, and c4 seem not to be controlled by one locus (Table 9). The high frequency of the phenotype c1c3c4 raised some doubt about whether c^1, c^3, and c^4 are controlled by the same locus. The occurrence of the phenotype c1c3 and -/- in the progeny of the mating, c^1c^3 x -/-, is evidence that c^1 and c^3 are at different loci (Table 10). The matings

Table 6. Segregation of b^1 and b^2

Deduced parent genotype[a] sire	dam	Number of: matings	progeny		Phenotype of progeny[a] b1	b1b2	b2	—	Probability of larger χ^2 value
b^1b^1	b^1b^2	9	67	Observed	24	43	0	0	0.01-0.025
				Expected	33.5	33.5	0	0	
b^1b^2	b^1b^1								
b^1/-	b^1b^2	7	35	Observed	17	14	2	2	0.025-0.05
				Expected	17.5	8.75	8.75	0	
b^1b^2	b^1/-								
b^1/-	b^2/-	3	21	Observed	8	2	7	4	0.25-0.50
				Expected	5.25	5.25	5.25	5.25	
b^2/-	b^1/-								
b^1b^2	b^2/-	4	26	Observed	5	7	9	5	
				Expected	6.5	6.5	13	0	
b^2/-	b^1b^2								

[a]The dash means not allotypes b1 or b2 or their alleles.

Table 7. Segregation of b^1 ignoring b^2

Deduced parent genotype[a] sire	dam	Number of: matings	progeny		Phenotype of progeny[a] b1	—	Probability of larger χ^2 value
$b^1/-$	x $b^1/-$	7	35	Observed	31	4	0.25
				Expected	26.25	8.75	
$b^1/-$	x $-/-$	7	47	Observed	22	25	0.50-
				Expected	23.5	23.5	0.75
$-/-$	x $b^1/-$						
b^1b^1	x $b^1/-$	14	101	Observed	101	0	
				Expected	101	0	
$b^1/-$	x b^1b^1						

[a]The dash means not allotype b1 or its allele.

Table 8. Segregation of b^2 ignoring b^1

Deduced parent genotype[a] sire	dam	Number of: matings	progeny		Phenotype of progeny[a] b2	—	Probability of larger χ^2 value
$b^2/-$	x $b^2/-$	4	26	Observed	16	10	0.10-
				Expected	19.5	6.5	0.25
$b^2/-$	x $-/-$	19	123	Observed	68	55	0.25-
				Expected	61.5	61.5	0.50
$-/-$	x $b^2/-$						

[a]The dash means not allotype b2 or its allele.

Table 9. Segregation of c1, c3, and c4

Parent phenotype[a]			Number of:		Phenotype of progeny[a]							
sire		dam	matings	progeny	c1	c3	c4	c1c3	c1c4	c3c4	c1c3c4	—
c1c3	x	c4	5	31	1	0	0	2	13	2	13	0
c1c3	x	c1c4	2	15	2	2	0	2	5	0	4	0
c1c3	x	c1c3c4	3	22	0	2	0	2	4	5	9	0
c1c3c4	x	c4	3	22	0	0	9	1	4	1	6	1

[a]The dash means not allotypes c1, c3, or c4.

Table 10. Segregation of c^1 and c^3 ignoring c^4

Deduced parent genotype[a] sire	dam	Number of: matings	progeny		Phenotype of progeny[a] c1	c3	c1c3	—	Probability of larger χ^2 value
c^1c^1	$c^3/-$	1	10	Observed	4	0	6	0	0.50-0.75
				Expected	5	0	5	0	
$c^1/-$	c^1c^3	4	25	Observed	14	6	5	0	0.50-0.75
c^1c^3	$c^1/-$			Expected	12.5	6.25	6.25	0	
c^1c^3	c^1c^3	4	29	Observed	7	11	11	0	0.25
				Expected	6.75	6.75	13.5	0	
c^1c^1	c^1c^3	1	9	Observed	5	0	4	0	0.75
				Expected	4.5	0	4.5	0	
c^1c^3	$-/-$	8	54	Observed	19	3	21	11	
$-/-$	c^1c^3			Expected	27	27	0	0	

[a]The dash means not allotypes c1 or c3 or their alleles.

$c^1/-$ x c^1c^4 and c^1c^4 x $c^1/-$ (Table 11) also produced the phenotype -/-, indicating that c^1 and c^4 are not alleles. On the other hand, the results given in Table 12 generally support the hypothesis that c3 and c4 are determined by a single locus.

Table 13 gives the segregation of c^1 ignoring c^3 and c^4. Apparently c^1 segregated in simple mendelian fashion. For the mating type, $c^1/-$ x -/-, the high chi square value may have been due to misclassifying genotypes of some of the parents. Three matings of c^1c^3 x -/- (Table 10) yielded 27 offspring all carrying c1, indicating at least one parent was a c^1c^1 homozygote. Since c^1 and c^3 were assumed to be alleles, the parents for the mating c^1c^3 x -/- were assumed to be $c^1/-$ and -/- (relative to c^1; in this particular case, the - for $c^1/-$ was c^3). If the above matings are deleted from Table 13, the ratio of c^1 to non-c^1 becomes 34:32, which is very close to agreement with the expected 33:33 ratio. The segregations of c^3 anc c^4 follow simple mendelian expectation also (Tables 14 and 15).

The c1 allotype seems to be the "extra" antigen in the c system. There are several hypotheses alternative to allelism at locus c for explaining the genetics of c1. First, this antigen could be controlled by an allele at locus a or b. However, since several individuals had the phenotype a1a2 b1b2 c1c3c4, c1 is evidently controlled by a locus

Table 11. Segregation of c^1 and c^4 ignoring c^3

Deduced parent genotype[a] sire	dam	Number of: matings	progeny		Phenotype of progeny[a] c1	c4	c1c4	—	Probability of larger χ^2 value
c^1c^1	c^1c^4	3	16	Observed	7	9	0	0	0.50-0.75
				Expected	8	8	0	0	
c^1c^4	c^1c^1								
c^1/-	c^1c^4	5	32	Observed	5	7	15	4	
				Expected	16	8	8	0	
c^1c^4	c^1/-								
c^1/-	c^4/-	6	25	Observed	6	2	12	5	0.025-0.05
				Expected	6.25	6.25	6.25	6.25	
c^4/-	c^1/-								
c^1/-	c^4c^4	4	21	Observed	0	2	19	0	0.005
				Expected	0	10.5	10.5	0	
c^4c^4	c^1/-								
c^1c^4	c^4c^4	6	37	Observed	0	17	20	0	0.50-0.75
				Expected	0	18.5	18.5	0	
c^4c^4	c^1c^4								

[a]The dash means not allotypes c1 or c4 or their alleles.

Table 12. Segregation of c^3 and c^4 ignoring c^1

Deduced parent genotype[a] sire	dam	Number of: matings	progeny		Phenotype of progeny[a] c3	c4	c3c4	—	Probability of larger χ^2 value
$c^3/-$	x $c^4/-$	3	22	Observed	7	5	6	4	0.50-0.75
				Expected	5.5	5.5	5.5	5.5	
$c^4/-$	x $c^3/-$								
$c^3/-$	x c^3c^4	4	25	Observed	8	4	13	0	0.01
				Expected	12.5	6.5	6.5	0	
c^3c^4	x $c^3/-$								
c^3c^4	x c^4c^4	5	32	Observed	0	15	17	0	0.75
				Expected	0	16	16	0	
c^4c^4	x c^3c^4								

[a]The dash means not allotypes c3 or c4 or their alleles.

Table 13. Segregation of c^1 ignoring c^3 and c^4

Deduced parent genotype[a] sire	dam	Number of: matings	progeny		Phenotype of progeny[a] c1	—	Probability of larger χ^2 value
$c^1/-$	x $c^1/-$	9	65	Observed	44	19	0.25-0.50
				Expected	48.75	16.25	
$c^1/-$	x $-/-$	17	93	Observed	61	32	0.005
$-/-$	x $c^1/-$			Expected	46.5	46.5	
c^1c^1	x $c^1/-$ or $-/-$	8	46	Observed	46	0	
$c^1/-$ or $-/-$	x c^1c^1			Expected	46	0	

[a]The dash means not allotype c1 or its allele.

Table 14. Segregation of c^3 ignoring c^1 anc c^4

Deduced parent genotype[a] sire	dam	Number of: matings	progeny		Phenotype of progeny[a] c3	—	Probability of larger χ^2 value
$c^3/-$	x $c^3/-$	6	30	Observed	24	6	0.50-0.75
				Expected	22.5	7.5	
$c^3/-$	x -/-	16	123	Observed	62	61	0.90-0.95
-/-	x $c^3/-$			Expected	61.5	61.5	

[a]The dash means not allotype c3 or its allele.

Table 15. Segregation of c^4 ignoring c^1 and c^3

Deduced parent genotype[a] sire	dam	Number of: matings	progeny		Phenotype of progeny[a] c4	—	Probability of larger χ^2 value
$c^4/-$	$c^4/-$	4	20	Observed	13	7	0.25-0.50
				Expected	15	5	
$c^4/-$	$-/-$	9	56	Observed	28	28	
$-/-$	$c^4/-$			Expected	28	28	
c^4c^4	$c^4/-$ or $-/-$	12	68	Observed	68	0	
$c^4/-$ or $-/-$	c^4c^4			Expected	68	0	

[a]The dash means not allotype c4 or its allele.

other than a and b. Secondly, phenogroups could be involved but the evidence is inconclusive. A third alternative would be linkage between loci and a fourth the presence of additional independent loci. Table 16 lists the results expected for the latter possibilities using the mating, c^1c^3 x -/- (Table 10), as an example. Either the third or fourth alternative could explain the observed segregation of c^1, c^3, and c^4, but the evidence does not favor either conclusively.

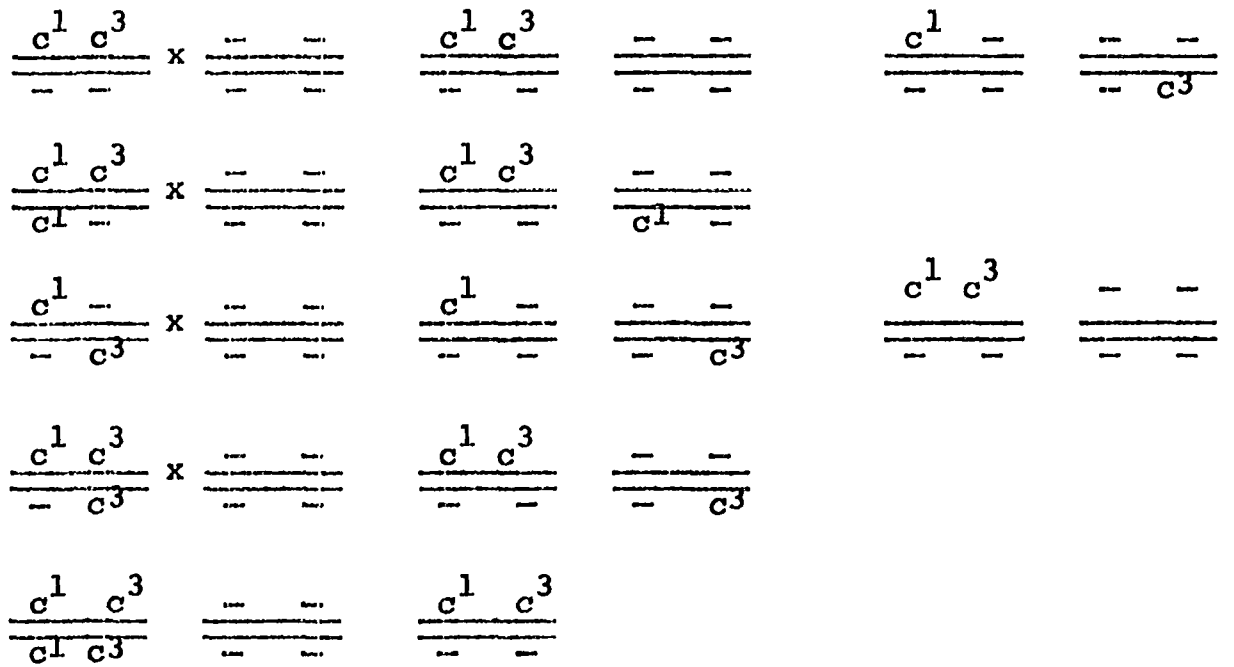
The distribution of the phenotypes for all of the genetically identified allotypes except c2 is given in Table 17 according to sex. The hypothesis that the allotypes are sex-limited is rejected.

Several groups of chickens were tested at least twice for the allotypes a1, a2, b1, c3, and c4 in order to check both the quality of the antisera and technique. The number of sera which failed to show a definite reaction in both tests represent discrepancies. The failure of all tests to be 100 percent reproducible (Table 18) was probably due to the titer of the particular antiserum sample used and perhaps to cross reactions. Both a1 and c3 were typed with the same antiserum. Because this reagent was not absorbed it was not mono-specific. The precipitation lines for these antigens in gel diffusion were usually distinctly separate. Occasionally, however, the two precipitation lines were not

Table 16. Expected segregation of c^1 and c^3 from the mating, c^1c^3 x -/-, assuming independence and linkage

Hypothesis	Chromosome makeup of parents	Chromosome makeup of progeny[a]	
		Non-cross over types	Cross over types
Independence	$\frac{c^1}{-}\ \frac{c^3}{-} \times \frac{-}{-}\ \frac{-}{-}$	$\frac{c^1}{-}\ \frac{-}{-}$, $\frac{c^3}{-}\ \frac{-}{-}$, $\frac{c^1}{-}\ \frac{c^3}{-}$, $\frac{-}{-}\ \frac{-}{-}$	
	$\frac{c^1}{c^1}\ \frac{c^3}{-} \times \frac{-}{-}\ \frac{-}{-}$	$\frac{c^1}{-}\ \frac{-}{-}$, $\frac{c^1}{-}\ \frac{c^3}{-}$	
	$\frac{c^1}{-}\ \frac{c^3}{c^3} \times \frac{-}{-}\ \frac{-}{-}$	$\frac{-}{-}\ \frac{c^3}{-}$, $\frac{c^1}{-}\ \frac{c^3}{-}$	
	$\frac{c^1}{c^1}\ \frac{c^3}{c^3} \times \frac{-}{-}\ \frac{-}{-}$		$\frac{c^1}{-}\ \frac{c^3}{-}$

Linkage



[a]The dash means not alleles c^1 or c^3.

Table 17. Test for independence between sex and allotypes

Allotype allele	Number of progeny		Males	Females	Probability of larger χ^2 value
a^1	137	Observed	63	74	0.25-0.50
		Expected	68.5	68.5	
a^2	100	Observed	51	49	0.90-0.95
		Expected	50	50	
b^1	134	Observed	71	63	0.50
		Expected	67	67	
b^2	84	Observed	43	41	0.90-0.95
		Expected	42	42	
c^1	86	Observed	41	45	0.75-0.90
		Expected	43	43	
c^3	58	Observed	30	28	0.75-0.90
		Expected	29	29	
c^4	121	Observed	59	62	0.75-0.90
		Expected	60.5	60.5	

Table 18. Consistency of precipitin test reactions

Allotype	Number of: Chickens tested	Reactions (R)[a]	Discrepancies (D)[b]	Consistency of test (%)[c]
a1	109	82	3	96
a2	48	34	2	94
b1	30	27	0	100
c1	94	63	4	94
c3	109	47	0	100
c4	151	131	15	89

[a]Number of sera which reacted in at least one of two tests.

[b]Number of sera which reacted in only one of two tests.

[c][(R-D)/R][100].

clearly separate for individuals carrying both a1 and c3 (see Figure 2a, Materials and Methods). The a2 antiserum seemed to be more sensitive to variable test conditions than the other reagents and the strength of the reactions varied somewhat. Hence, the reactions for a2 were not as repeatable as for some of the others. The b1 antiserum reacted strongly and clearly and only technical errors could cause discrepancies.

Two different antisera, harvested on different dates, were used for typing c1. In 40 chicken sera showing 30 reactions with the first sample, four discrepant reactions were noted (87 percent consistent). Tests of the remaining

54 sera, 33 reactions, against a second sample revealed no discrepant reactions. The first sample reacted weakly and the second strongly. Thus, discrepant reactions in this case were probably due entirely to low titer antiserum. The same problem was also at least partly responsible for the c4 discrepancies. Two different samples of antiserum were used to test for c4; the first reacted weakly while the second reacted strongly.

Antiserum Production

A total of 247 chickens were injected. The serum from 42 recipients reacted with the respective donor serum in gel diffusion. Thirty of the latter reacted with normal reference sera. In most cases, we have not definitely ascertained whether new antisera duplicated standard antisera. Therefore, antigenic designations of the antisera listed in the tables in the Results section are only tentative unless otherwise indicated.

Immunizations in 1968

Five recipient sera gave weak to medium strength reactions when tested against their donor serum (Table 19). Two antisera (A5801, A5998) sometimes did not react with the donor serum placed in the center well, but reacted only with the donor serum placed in an adjacent peripheral well. This was probably because the peripheral wells were closer to

Table 19. Antiserum production in 1968

Donor wingband no.	Recipient sera			Antisera produced[b]
	No. tested	No. which reacted with donor serum	No. which reacted with nrs[a]	
5822	4	1	1	A5811
5823	3	2	2	A5801
				A5825-C1
5833	4	1	1	A5806-Ax2
5998	3	1	1	A5998
Total	14	5	5	

[a]Normal reference sera; new antisera were tested against at least nine.

[b]Allotypic designations are tentative.

each other than they were to the center well and the antisera had relatively low titers.

Immunizations in 1969

Series I - No anti-allotype antisera were produced by the five subcutaneous injections. Furthermore, the intravenous injection of B. abortus - anti-B. abortus complex failed to induce detectable antibody. All samples were retested and the results were again negative.

Series II - Only two of 27 recipients (A18874, B2147) produced sera which reacted with donor serum. The precipitin in B2147 was not directed against an identified allotype, as

shown by its failure to react with known allotype normal reference sera.

Series III - The success of antiserum production from five injections, and the booster injection after the rest period, is shown in Table 20. Serum from S7936 reacted strongly with donor serum 5801 in less than twelve hours. The balance of antisera gave medium strength reactions.

Since the sera from the recipients of donor 5801 did not react with normal reference sera, they do not contain antibodies against the identified allotypes. These new antisera may contain a precipitin against an unknown allotype or against an idiotype. To test the latter possibility, the new antisera were tested against samples of Brucella-absorbed and unabsorbed anti-Brucella serum from donor 5801. The antigenic specificity of 5801 immune serum was changed by absorption (Figure 7). For example, the spur formed in the reaction nearest the GW18002 well indicates that absorption removed a cross reacting determinant. The intersection of the other two precipitation lines around GW18002 shows that two different antigenic determinants, perhaps an allotype and an idiotype, were present in the unabsorbed donor serum. The precipitation lines for S7877 and S7981 did not clearly intersect. One precipitin in these new antisera (except S7936) was directed against a common determinant in the absorbed serum (Figure 8).

Table 20. Antiserum production in series III, 1969

Donor wingband no.	Recipient sera				Antisera produced
	No. tested	No. which reacted with donor serum before rest period	No. which reacted with donor serum after rest period	No. which reacted with nrs[a]	
5801	4	3	4	0	S7877, S7936, S7981, GW18002
5811	6	5	6	6	S7447, S7874, S7905, S7906, S7964, GW17985
5822	5	0	1	0	S7345
5939	5	1	—[b]	1	S7938
5952	1	0	—[b]	0	
6003	4	1	1	not yet tested	S7883
8367	4	0	1	1	S7870
20060	4	0	—[b]	0	
Total	33	10	13	8	14

[a]Normal reference sera; new antisera were tested against at least nine.

[b]Not given a booster injection after the rest period due to lack of donor serum.

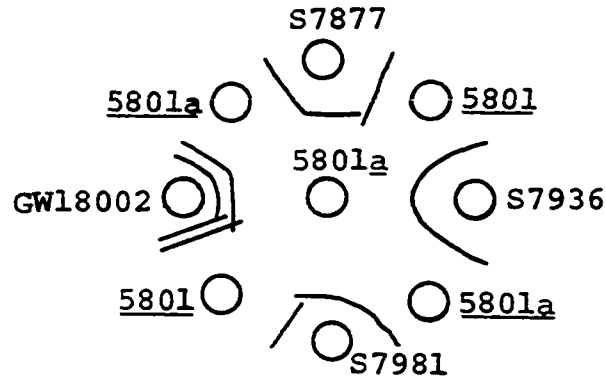


Figure 7. Tests of new antisera against Brucella-absorbed (5801a) and unabsorbed (5801) donor serum.

Figure 8. Tests for common specificities in new antisera using Brucella-absorbed (5801a) and unabsorbed (5801) donor sera.

Recipient antisera for donor 5811, and one for donor 5822 (S7345), were also tested against Brucella-absorbed and unabsorbed donor sera (Figure 9). Absorption changed the antigenic specificity of the donor sera in these cases also. Since the new antisera reacted with normal reference sera, they may carry precipitins directed against known allotypes in addition to a possible antibody against an idiotype.

Series IV - Only two precipitins were detected out of 39 chickens injected. Unfortunately, the recipients which produced the antisera (W14191, WG17580) died after the fifth injection. Since the recipient sera were tested using the pattern shown in Figure 4b (Materials and Methods), some antisera may have been missed. None of the antisera were retested.

The reactions for W14191 were of medium strength, while those of WG17580 were strong. The latter contained a precipitin against c1 and was mono-specific; W14191 may have contained an antibody against c1 also, but this has not yet been confirmed.

Series V - Sixteen antisera were made in series V, seven of which reached a detectable level before the fourth injection (Table 21). When the recipients were given a rest of more than two weeks between injections, the number of antisera was increased to 16. Those which failed to react with reference sera evidently did not contain precipitins against

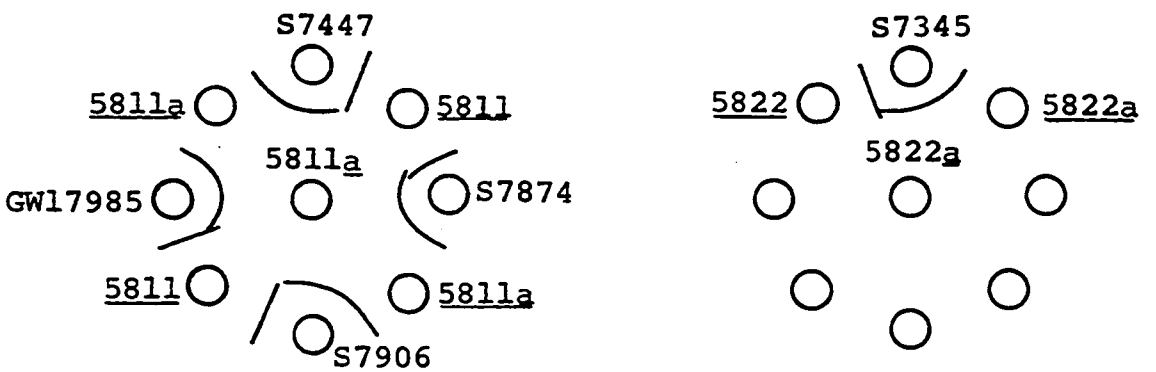


Figure 9. Test of new antisera against Brucella-absorbed (5811a) and unabsorbed (5811) donor serum.

Table 21. Antiserum production in series V, 1969

Donor wingband no.	Recipient sera: No. tested	No. which reacted with donor serum before inj.4	No. which reacted with donor serum after inj. 4	No. which reacted with donor serum after inj. 5	No. which reacted with nrs[a]	Antisera produced[b]
2412	5	0	0	0		
2417	4	0	1	1	1	I9-59
2419	5	0	0	0		
2439	4	0	0	0		
2445	5	0	1	2	2	I9-86 GH-265-c4
2457	6	0	0	0		
2498	6	0	0	0		
18875	4	0	1	1	1	I9-74-c4
18879	7	1	2	3	3	GH-272,A18870, A18884
18898	8	5	8	6/7	4	GH-4,I9-83, I19-203,GH-352, I19-697,A18865, A18897,A18900
18902	4	0	0	0		
18906	5	1	1	1	0	A19079
Total	63	7	14	14	11	16

[a]Normal reference sera; new antisera were tested against at least nine.

[b]Allotypic designations are tentative.

known allotypes.

Five chickens in series V were recipients of previous injections. Although one (A18897) produced a precipitin against the donor serum 18898, there was no clear evidence that previous injections had any effect on antibody formation in this series.

The first six donors in Table 21 (2412-2457) were immunized against B. abortus intravenously for three weeks, while the second six were immunized by subcutaneous injections over several months. The former induced precipitin production in three of 29 recipients injected, while the latter induced formation in 13 of 34 recipients. Thus, immunization against the carrier antigen subcutaneously appeared to be more effective than intravenously. However, because the two groups of donors represented different subpopulations, the route of injection and subpopulation origin may have been confounded. Considering all injection series in this project, the subcutaneous route seems to have been more effective only when donors were from subpopulation A-b (Table 22).

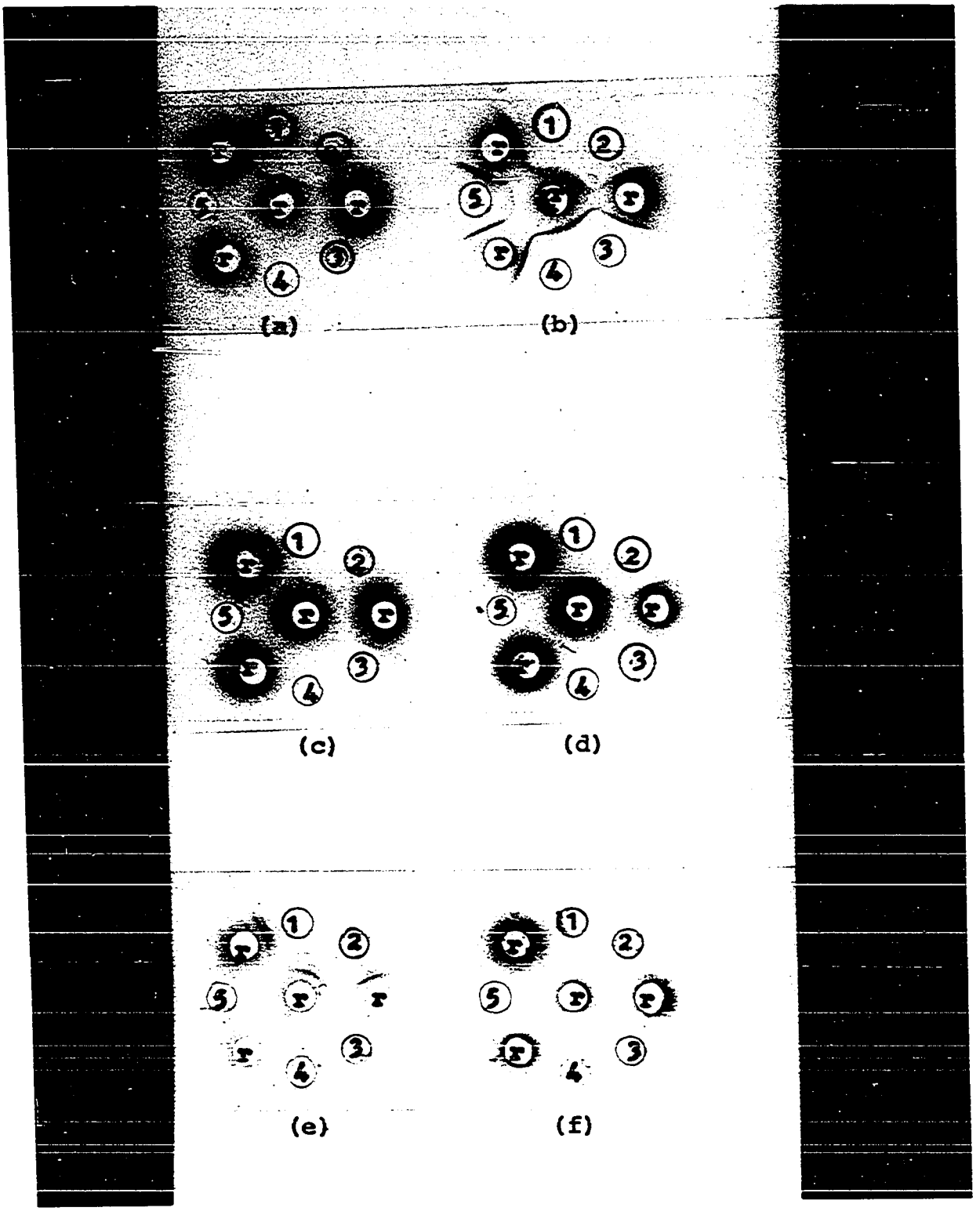
Since a few antisera produced weak reactions, two attempts were made to increase reaction strength. First, a sample of GH-265 (Table 21) was lyophilized, resuspended to one-fourth the original volume, diluted 1:2, 1:4, 1:8, and 1:16, and the undiluted, diluted, and unlyophilized samples

Table 22. Effect of route of immunization of the donor on precipitin production

Donor		Recipients		
Origin	Route of immunization	No. tested (n_t)	Antisera produced No.	Antisera produced Percent of n_t
A-a	Intravenous	60	3	5
	Subcutaneous	19	1	5
A-b	Intravenous	34	6	18
	Subcutaneous	63	26	41
A-c	Intravenous	20	1	5
	Subcutaneous	28	2	7

were tested against normal reference sera. All dilutions of the lyophilized sample reacted; the undiluted lyophilized sample reacted more strongly and rapidly than the unlyophilized sample, as expected. In the second attempt to produce stronger reactions, samples of serum from donors 5801, 5811, and 5822 (Table 20), absorbed with B. abortus, were diluted 1:2, 1:4, 1:8, and 1:16. The undiluted and diluted samples were then tested against the recipient antisera (Figure 10). Usually, the antisera reacted only with the undiluted donor sera. Those which gave more precipitate with the undiluted samples also reacted with some of the dilutions. Apparently, the undiluted samples had nearer the optimum concentration of antigen.

Figure 10. Tests of new antisera against undiluted and diluted samples of donor serum. Undiluted donor serum (u) was placed in well number 1; the dilutions 1:2, 1:4, 1:8, and 1:16 were placed, respectively, in wells 2, 4, 5, and 7. The new antiserum (r, recipient serum) was placed in the remaining peripheral wells and in the center well. The tests were: (a) Donor 5822 against recipient S7345; (b), (c), (d), (e) Donor 5801 against recipients S7936, S7981, S7877, and GW18002, respectively; (f) Donor 5811 against recipient S7905.


(a)
(b)
(c)
(d)
(e)
(f)

Miscellaneous injections

The two turkeys which were injected produced anti-chicken antibodies. To determine whether the turkey sera also contained specific anti-allotype antibodies, a normal serum was titrated against the antisera (alpha procedure) in a tube precipitation test, and the resulting supernatants were tested for antibodies against allotypes. Donor 18903 for turkey number 1 had the phenotype a1 b2 c1c3c4; hence, theoretically, if turkey number 1 serum was absorbed with serum having the phenotype b2 c1c3c4, an a1 reagent might be made. However, the turkeys would not necessarily produce antibodies against all allotypes. Therefore, serum lacking all of the known antigens present in the donor was chosen for the titrations. Two hours after setting up the test, the titers of the normal serum were 1:128 for the turkey number 1 antiserum and 1:32 for the turkey number 2 antiserum. When the supernatants were tested against the donor serum in gel diffusion, no reactions appeared for the lower dilutions where the most precipitate formed in the tube test (Figures 11 and 12). These results seem to indicate that when precipitation was detectable in tubes, no antibody against allotypes remained in the supernatant. At least, antibody was not present in sufficient quantity to be detectable by double diffusion in gel.

Eight line HN chickens were injected with protein pre-

Figure 11. Gel diffusion test of supernatants from tube precipitation test - turkey no. 1, donor (D) 18903. The antigen (Ag) control consisted of antigen plus saline; the antibody (Ab) control was turkey no. 1 antiserum plus saline.

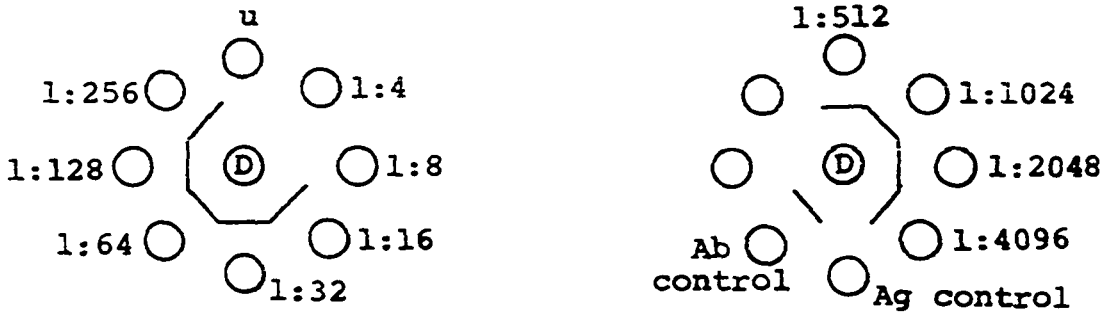


Figure 12. Gel diffusion test of supernatants from the tube precipitation test - turkey no. 2, donor (D) 2437. The antigen (Ag) control consisted of antigen serum plus saline; the antibody (Ab) control was turkey no. 2 antiserum plus saline.

cipitated with sulfosalicylic acid. Two antisera carrying the same specificities were produced. Both showed two precipitation lines when tested against normal reference sera. Evidently neither specificity is directed against any genetically identified allotypes.

From line GH chickens injected with egg yolk extract, one anti-allotype antiserum was made. Preliminary testing against normal reference sera indicated that the antibody was not directed against any of the identified antigens.

Effect of Donor and Recipient Origin on Antiserum Production

Only the data collected for the injections of 1968 and series III and V, 1969 are considered here. The antisera produced in the remaining injections series were so few that they were omitted from this section.

Injections within and between the allotype subpopulations seemed not to be effective in producing anti-allotype sera (Table 23). Only 18 percent of the recipients produced antisera compared with 48 and 40 percent of the non-inbred and inbred groups, respectively. However, the allotype subpopulation origin of the donor may have been the most important factor (Table 24). Chickens from A-a and A-c induced antiserum production in only seven and six percent of their recipients, respectively. Chickens from A-b, on the other hand, induced antibody formation in 56 percent of the

Table 23. Antiserum production in the three major recipient types

No. of donors	Recipient sera Origin	No. tested (n_t)	Reactions with donor serum No. (n_t)	Percent (n_r/n_t)(100)
20	A-a,b,c	66	12	18
7	non-inbred	29	14	48
10	inbred	25	10	40

Table 24. Relationship between donor and recipient origin and antiserum production

Donor Origin	Donor No.	Recipient sera Origin	No. tested (n_t)	Reactions with donor serum No. (n_t)	Percent (n_r/n_t)(100)
A-a	7	A-a	27	0	
	1	non-inbred	4	1	
	5	inbred	12	3	
Total	13		43	4	7
A-b	1	A-a	1	0	
	7	A-b	23	11	
	3	non-inbred	15	11	
	5	inbred	13	7	
Total	16		52	29	56
A-c	1	A-a	1	0	
	4	A-c	14	1	
	3	non-inbred	10	2	
Total	8		25	3	6

recipients. When the birds from A-a and A-c were selected as recipients for donors from the same subpopulation (i.e., A-a injected into A-a, A-c into A-c) only one antiserum of 41 possible was developed. However, when 26 recipients from other populations (inbred and non-inbred) were used, six antisera were made. Origin of the recipients in the case of A-b donors apparently had little effect on the outcome. These results suggest that the immunogenicity of the allotype antigens in the chickens of A-a and A-c was low relative to that of the allotypes in A-b, at least for the antigens against which antibodies were produced.

The number of antisera for each donor that induced antibody formation in at least one recipient is presented in Table 25. Eleven donors failed to induce any precipitin production in 43 recipients. For the remainder, 46 percent of the recipients produced antisera which reacted with the donor serum. Donors from A-a, A-b, and A-c induced precipitin production in 39, 63, and 18 percent of their recipients, respectively.

Pedigree relationships between donor and recipient were apparently not too important in determining whether or not an antiserum was made, when the donor was from A-b (Table 26). Nine antisera in 20 chickens were made when the recipients were full- or half-sibs of an A-b donor and 10 out of a possible 29 were produced when the recipients were unrelated

Table 25. Number of antisera produced, by donor[a]

Donor		Recipient sera		
Origin	Wingband no.	No. tested (n_t)	Reactions with donor serum	
			No. (n_t)	Percent (n_r/n_t)(100)
A-a	2417	4	1	25
	2445	3	2	67
	8367	4	1	25
			ave.	39
A-b	5801	4	4	100
	5811	6	6	100
	5822	9	2	22
	5823	9	4	44
	5833	3	1	33
	18875	4	1	25
	18879	8	4	50
	18898	8	8	100
	18906	5	2	40
			ave.	63
A-c	5939	10	1	10
	6003	7	2	29
			ave.	18
	Total	84	39 ave.	46

[a]Includes only those donors which induced antibody formation in at least one recipient.

Table 26. Effect of relationship between donors and recipients on antiserum production

Donor		Recipient sera			
		Full- or half-sibs of donor		Unrelated to donor[a]	
Origin	Wingband no.	No. tested	No. which reacted with donor serum	No. tested	No. which reacted with donor serum
A-a	2417	2	0	2	1
	2419	2	0	2	0
	2439	2	0	3	0
	2445	1	0	2	2
	2457	6	0	0	0
	2498	6	0	0	0
		19	0	9	3
A-b	5822	4	1	5	1
	5823	3	2	6	2
	5833	2	1	0	0
	18874	1	0	3	0
	18879	4	2	5	1
	18898	2	2	6	6
	18902	2	0	2	0
	18906	2	1	2	0
		20	9	29	10
	Total	39	9∼23%	38	13∼34%

[a]Recipients which were from a line or subpopulation different from that of the donor.

to the donor. On the other hand, no antisera were made by injecting serum from A-a donors into full- or half-sib recipients, while three of nine non-sib chickens produced antisera. Hence, the overall difference in antiserum production (23 versus 34 percent) between the two groups (full- or half-sibs of the donor versus those unrelated to the donor) was due mainly to the failure of the A-a donors to induce antibody formation in their sib recipients.

Effect of Allotype Antigen Dose and Injection Frequency on Antibody Response

Bleedings of recipients following each injection occasionally failed to yield serum in which precipitin was detectable even when the antibody was detected in serum from a previous bleeding of the same chicken. Failure to detect precipitin when it should have been present was considered, at first, to be due to improper gel diffusion technique. However, testing all bleedings on the same gel slide gave the same results as testing the sera separately after each bleeding.

After a chicken began producing anti-allotype antiserum, injections were made at monthly instead of bi-weekly intervals. However, in 1968, injections were continued every two weeks because the initial tests did not definitely show the presence of antibody. Retests showed that five chickens were making anti-allotype serum, but all sera were negative

after the final injection. It seems that the birds may have been injected too often, causing suppression or paralysis of the immune mechanism.

Both antigen dose and frequency of injection differed from the basic procedure in series III and V, 1969 (steps 4 and 9 of the basic procedure in Materials and Methods). Increased time between injections in both series increased the number of antisera produced (Tables 20 and 21). In series III, the antigen dose was increased above the basic dose for the first five injections but the rest period was given simultaneously with a decrease back to the basic dose. Hence, the increase in number of antisera cannot be attributed conclusively to either antigen dose or injection frequency. However, the series V results indicate that the frequency of injections was the most important factor. First, the dose was four to five times the basic dose for the first four injections, but the four-week delay between the third and fourth injections led to a gain of eight antisera (Table 21). The basic dose was used for injection 5 simultaneously with a five-week rest period, leading to a gain of only one antiserum. Secondly, because donor 18898 had a relatively low titer of anti-Brucella antibody, only the basic dose was used. The increased time between injections 3 and 4 increased by three the number of antisera from the recipients of this donor.

DISCUSSION

About 17 percent (40/239) of the recipients in this project produced antisera using Brucella abortus as a carrier antigen and subcutaneous injections with adjuvant. David (1966) made antisera against allotypes in 15 percent (25/165) of his recipients using a similar technique. Thus, immunizations in the two projects were about equally effective. Antisera were produced within four to six weeks of the initial injection of donor globulin. Other workers have found that more prolonged immunizations of greater than three months were necessary to induce a response when routes of injection were other than subcutaneous without adjuvant (Skalba, 1966; McDermid et al, 1969). The fraction of recipients producing antibodies in each injection series ranged from 4/47 to 10/29 for David (1966) and 0/29 to 14/32 for the present project. The variation in response may be due to a number of factors, particularly the widely different genetic backgrounds of the donors and recipients used.

Low Success in Producing Allotype Antisera in Chickens

Several factors may contribute to the low frequency of anti-allotype antisera produced in chickens. Some of these are: (1) individual variation among donors and among

recipients in the ability to induce antibody formation or to respond to antigenic stimulation, (2) competition between antigens for the production of antibody, (3) inability to detect antibody that is present, (4) maternally-induced tolerance of recipients to allotype antigens, and (5) presence of subtype variants.

Donors could differ in immunogenicity because of differences in concentration of serum allotypic determinants. In rabbits, b^4b^5 heterozygotes were found by Dray and Nisonoff (1963) to have different levels of the two allotypes in the serum. Hence, different titers of allotypes could help determine whether or not an antiserum is produced, depending on the allotype for which the donor differs from the recipient. Antigens of high concentration might be more immunogenic than those of lower concentration. Ability to induce antibody formation against different allotypic determinants varies in rabbits (Kelus and Gell, 1967). Oudin (1960a) found evidence that this is due to differences in antigen concentrations.

Recipients differ in ability to respond to antigenic stimulation. Genetic differences between host individuals may cause differences in disease resistance. Mice show genetic variation in resistance to Bacillus piliformis and to Salmonella typhimurium and guinea pigs show single gene effects in resistance to Salmonella cholerasuis (Gowen, 1960).

Genetic influences have also been found for susceptibility to virus infections in chickens (Stone, 1967; Vogt, 1967; Stone et al, 1970). Similarly, genetic variation in response to antigens of non-pathogenic origin has also been reported. McDevitt and Sela (1965) immunized two strains of mice with a synthetic polypeptide. The difference in response to this antigen between the two strains seemed to be due to a single gene. Similar results were reported by Pinchuck and Maurer (1965) who used a different polypeptide. At least three genes seemed to be responsible for variation in response between mouse strains immunized with sheep red blood cells by Playfair (1968). Thus, genetic differences between individual chickens probably affects production of antiserum against allotype antigens also.

Competition between antigens for the production of antibodies has been reported in chickens by Abramoff and Wolfe (1956), in rabbits by Adler (1957), in guinea pigs by Harel et al. (1970), and in mice by Kawaguchi (1970). Simultaneous injection of two antigens can cause production of low-titer antibodies directed against one or both antigens. In the present study, allotypic antigens could have competed with each other or with the carrier antigen Brucella abortus.

When two antigens are introduced into an animal at the same time, antibody formation against one determinant may

be depressed but formation against a second determinant may be enhanced (Siskind and Benacerraf, 1969). A carrier antigen could selectively cover certain determinants leading to depression of antibody formation against the covered antigen and possibly to enhancement of antibody production against a second antigen (Schierman et al, 1969). Thus, we might be producing antibodies consistently against the same allotypic determinants in chickens.

Low success in making antisera might also be due to simple failure to detect antibody when it is present. Failure to detect antibody, in turn, could be due to low antibody titers, to substances in the serum which inhibit precipitation, or to the production of univalent antibodies. All antisera in this study had low antibody titers as determined by gel diffusion. The antiserum which gave the strongest reactions had a titer of only 1/64. Hence, some chickens probably produced antibodies which were not detectable by gel diffusion. Inhibition of precipitation has been reported by several workers. Feinberg (1958) injected human serum albumin subcutaneously in Freund's complete adjuvant and found, in addition to production of homologous antibody, production of one that inhibited precipitation of the soluble antigen-antibody system. Similar results were found by Rodkey and Freeman (1970). In the present study, antibodies made by the recipients against Brucella abortus could have

inhibited precipitation of the allotype antigens by their homologous antibody. Repeated immunizations with small quantities of antigen can lead to the production of predominantly univalent antibodies (Raffel, 1961). Because evidence concerning antibodies having only one combining site in chickens has been inconclusive (Grey, 1969), this possibility needs to be explored further.

Maternal allotypes may be transferred to offspring in chickens as shown by Khattab and Craig (1970b). The transferred allotypes may induce tolerance to those originating in the offspring. Natural tolerance to allotypes apparently does not exist in rabbits (Gell and Kelus, 1966). However, immunization of pregnant does against an allotype in the homozygous male parent evidently depresses the expression of that allotype for several months in the offspring (Dray, 1962; Mage and Dray, 1965; Mage et al, 1967). When the allotype finally appeared in the offspring, it was at a very low level compared to that in offspring of non-immunized mothers. Maternal allotypes might have a similar effect in chickens that maternal antibodies against allotypes seem to have in rabbits.

Subtypes, found for blood group antigens in humans and cattle (Miller, 1958), can affect antiserum production. When, for example, two subtypes are present in the donor, production of antibodies against one may be inhibited by

the presence of the other (Miller, 1970)[1]. Whether or not subtypes are involved in the genetics of allotypes is apparently unknown.

Precipitation Reactions

Strength of reactions of recipient antisera seemed to vary during the course of immunization. Amount of precipitate formed in gel diffusion depends on the size of the antigen and antibody molecules and their diffusion constants, as well as the ratio of antigen concentration to antibody concentration (Carpenter, 1965). If the latter ratio is too far from optimum, a reaction will not be detectable.

The amount of precipitate formed in gel diffusion also depends on the titer of the antiserum. Antibody titer during the immunization schedule depends, in turn, on several factors. Rodkey and Freeman (1970) found that both the level and precipitating efficiency of anti-bovine serum albumin antibody decreased late in immunization. Low primary doses of antigen tend to cause low antibody titer initially but high titer later in the response (Goidl et al, 1968; Siskind and Benacerraf, 1969). The reverse seems to

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[1]Miller, Wilmer J. Department of Genetics, Iowa State University, Ames, Iowa. Private communication. 1970.

be true if a large primary dose is given. These observations were based on a single injection of antigen emulsified in Freund's complete adjuvant. Thus, with a large primary dose, there was initially a large amount of antigen present when the antibody titer was high and later a lower antigen concentration with still high antibody titer. In our project, at least some of the antigens could have been in relatively high concentrations so that early in the immunizations detectable antibody was produced. Later, with the antigen concentration being maintained at a relatively high level in the recipients, the immune mechanism may have become paralyzed. According to Sterzl(1966) a large initial dose of antigen may be followed by a high primary response, but a booster injection is followed by a low secondary response.

Amount of precipitate formed in gel diffusion tests can also be affected by the affinity of an antibody (strength of interaction between an antibody and an antigenic determinant) for its respective antigen (Goidl et al., 1968; Siskind and Benacerraf, 1969). Affinity, in turn, varies with time in the immunization period, with antigen dose, and with the physical state of the antigen. Following a large dose of antigen, low-affinity antibody may be found late in the response after the antibody titer has decreased. The reverse seems to be true for low primary doses. Booster in-

jections tend to give relatively high affinity antibody but this may decrease somewhat as the time interval between the primary and second injections is shortened. Each antigen has its own optimum dose, relative to affinity, which differs for each species of animal. Finally, soluble antigens tend to lead to production of low-affinity antibody. A combination of these factors may have been involved in the present study.

Disappearance of antibody during the course of immunization could be due to suppression of antibody formation caused by the antibody itself. Antibody introduced into antigen-treated animals can suppress the immune response when the antibody is injected either at the same time as the antigen or after the antigen (Coe and Salvin, 1964; Uhr and Möller, 1968; Möller, 1969; Siskind and Benacerraf, 1969). Furthermore, suppression depends on the continued presence of the antibody in the animal (Uhr and Möller, 1968). Thus, in the present study, any antibody produced after one injection could suppress further antibody production if the antigen was reintroduced too soon after the initial response.

Antisera against allotypes in rabbits are produced fairly readily. The most important causes of differences between rabbits and chickens in producing antisera are the inherent species differences. Chickens seem to be unique in certain immunological characteristics, the major one being

the necessity for high salt concentration (above seven percent) in precipitation tests. Part of the difference in amount of precipitate formed at high versus low salt concentration is due to coprecipitation of a lipoprotein macroglobulin (Grey, 1969). The coprecipitation is greater at high percent salt and with aged serum. The lipoprotein component, in itself, adds to the precipitate and also may cross-link soluble complexes into larger insoluble complexes (Grey, 1969). Occasionally this could lead to false positive results, both in the case of typing chickens for allotypes and in testing recipient sera for anti-allotype precipitins. In the latter case, the apparent number of allotypic determinants might be larger than the actual number making allotypic systems in chickens appear to be more complicated than they really are. Also, coprecipitation could lead to the "hazy" or blurred appearing reactions which were sometimes found in this study. Circumstantial evidence for this was the clearer reactions found when recipient sera were tested against Brucella-absorbed donor serum compared to the reactions obtained in tests of recipient sera against unabsorbed donor serum. Brucella abortus antigen was found in our laboratory to combine non-specifically with lipids in chicken serum; perhaps it also combines non-specifically with lipoprotein.

Improvement of Methods to Produce Allotypic Antisera

Several techniques might be tried to improve the effectiveness of making allotypic antisera in chickens. First, doses of donor globulin smaller than those used in this study might be more effective. Secondly, gram-negative bacteria which release endotoxins could be tried as the carrier antigens, although very large doses might be necessary to produce an adjuvant effect of the endotoxins (White, 1967). The ability to determine beforehand which donors induce antibody formation in recipients would greatly increase the efficiency of antiserum production. A gene dosage effect may influence the concentration of allotypes in serum (Dray and Nisonoff, 1963). Hence, if antigens of high concentration are more immunogenic than those of low concentration, then allotypic homozygotes would make more effective donors than heterozygotes.

Donor sera pooled from several chickens might be more effective in producing anti-allotype precipitins than serum from a single individual. In particular, serum samples from, say, three chickens with the same allotypic phenotype could be pooled. The probability that a particular antigen in the pooled serum would be sufficiently antigenic to induce antibody formation would be greater than if only one donor was used.

In an attempt to find more efficient ways to utilize

inbreds for antiserum production, five lines were surveyed (Table 27). Some of these lines might have special value either as donors or recipients. For example, only the Spanish line seems to carry the allotype a1 while none carry c3. Hence, the Spanish line chickens could be used as a1 donors or c3 recipients; other lines could be used as recipients of either a1 or c3. The Leghorn line GH might be used as recipients for most of the identified allotypes.

Table 27. Survey of inbred lines for allotypes[a]

Line	Number of sera which reacted with:						
	a1	a2	b1	b2	c1	c3	c4
9	0	7	1	1	7	0	1
19	0	7	6	7	5	0	5
GH	0	6	0	2	0	0	6
Sp	5	5	4	6	5	0	7
HN	0	6	7	7(?)	2	0	3

[a]Seven serum samples were tested per line.

Whole normal serum injected subcutaneously with Freund's complete adjuvant has been used with some success in making allotypic antisera in chickens. David (1966) detected one precipitin in nine recipients immunized in this manner. The technique might appear inefficient, but the particular donor and recipient combinations may have been important in

determining whether or not an antibody was produced. Because this method does not require a carrier antigen, it is simpler to use than immune serum. Also, it sidesteps the complication of antibodies being produced against a carrier antigen. Thus, further experimentation with normal donor serum might prove fruitful. Injections, with adjuvant, of gamma globulins precipitated with alum or ammonium sulfate might also be tried. The latter technique has been utilized successfully in rabbits (Leskowitz, 1963).

Some of the allotypic determinants in chickens have been detected in egg yolks (Skalba, 1966; David, 1969[1]). Globulin extracted from egg yolks could probably supplement serum for making anti-allotype antiserum. This would be particularly useful if a hen carried an allotypic determinant which was highly antigenic, since a large supply of donor globulin could be built up in a relatively short time if the hen was in production. Furthermore, since hens usually have less blood than cocks, they should also have less serum.

Not all known allotypes have been detected in egg yolks. This may be due either to differences in concentration between antigens in the yolk or to differences in titer between anti-allotype reagents. Regarding the latter, only

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[1]David, C. S. University of Michigan, Ann Arbor, Michigan. Private communication. 1969.

the b1 antigen has been found in the yolk in our laboratory; significantly, the b1 antiserum also has the highest titer. Using yolk globulins to produce serum containing antibody against a non-b1 allotype shows that the yolk may contain other allotypes.

Stronger reactions in gel diffusion might be produced if the antiserum well is filled two or three times, say, once every two hours for six hours after the test is set up. Lyophilization of low titer antisera followed by resuspension, for example, to one-fourth the original volume, should also increase reaction strength although this would reduce the amount of antiserum. More gel on each slide, say, four or five ml. instead three ml., might also help to increase the amount of precipitate. On the other hand, development and standardization of a more sensitive allotype test might be the best solution to this problem.

Maintenance of a Segregating Allotypic Population of Chickens

The value of the population which has been developed depends largely on the success in replenishing the supplies of the standard antisera. The population can be used to make new anti-allotype antisera and as a source of reference sera for checking the specificities of new antisera.

Although the supplies of two reagents (b2, c2) have been exhausted, and another (a2) nearly so, matings could be set up such that segregation for these alleles would continue. For example, although we cannot type for b2, the mating $b^1b^1 \times b^2b^2$ would allow segregation for both b^1 and b^2 for at least two more generations; in the second generation the mating would be $b^1b^2 \times b^1b^2$. Alternatively, the population could be maintained as single locus non-segregating sublines from homozygous matings such as $b^1b^1 \times b^1b^1$. This would insure the maintenance of alleles in the population until such time that specific antisera can be reproduced. Because the c2 antigen has been disregarded for the past two generations, the allele c^2 is assumed lost.

Some of the new antisera may contain antibodies against allotypic determinants which are controlled by alleles at identified loci. Matings could be made whereby unknown alleles might be segregating with known alleles. The parents and offspring would be typed with both new and standard antisera. Offspring from matings which show evidence for new alleles at known loci would then be mated appropriately to test for the expected genetic ratios. For example, from the mating $y^1y^2 \times y^3y^4$ the absence of the phenotype y1 y2 y3 y4 would be evidence that some of the y's are allelic.

The specific goal remains to produce a population

segregating only for known allotypic alleles. Such a population could then be tested for possible relationships between allotypes and performance traits. In addition, if the population is typed for blood groups, linkage tests could be made between allotypic genes and blood group genes.

Possible Applications for Chicken Allotypes

Allotypes in chickens might be used as genetic markers for both immunologic and immunogenetic experiments. First, one could study the relative ability of cells of different lymphoid organs, such as the bursa of Fabricius, to produce an allotype when passively transferred from a chicken carrying the allotype to one not previously carrying it. According to results of a recent study by Khattab and Craig (1970a), the production of an allotype can be passively transferred to chicks by implanting spleen cells or lymphocytes from an adult donor to day-old chicks; this ability lasted for several months. Experiments might be tried in which the donor cells are taken from embryos or day-old chicks instead of from adult chickens. The question is whether neonatal transplanted donor cells would produce the donor allotype for a longer period of time in the recipients than do transplanted mature cells.

The suppression of allotypes might also be studied experimentally. For example, an inseminated hen could be

immunized against an allotype present in the homozygous sire to test whether the expression of the allotype in the offspring would be suppressed as apparently happens in rabbits according to Mage et al. (1967). Such an experiment in chickens would be unique in that the mother and embryo would be separated.

Additional basic studies which could be conducted include determination of the relative quantities of antibodies and allotypes and of the relative quantities of one allotypic determinant to another in serum and egg yolks. Also, it would be of interest to know which cells, immunoglobulin molecules, and chains carry the different allotypic determinants in chickens.

Of special interest to the poultry industry might be a study of the possible interrelationships between the bursa of Fabricius, serum protein allotypes and avian lymphoid leukosis. Bursectomy seems to prevent lymphoid leukosis (Petersen et al., 1964). Furthermore, certain allotypes may disappear after the onset of lymphoid tumor growth (David and Fletcher, 1970). The question is whether certain relationships between these factors could be useful to the poultry industry to better control lymphoid leukosis.

PART II. EFFECTS OF THE SEX-LINKED RECESSIVE DWARF GENE, dw, ON QUANTITATIVE TRAITS IN THE FOWL

REVIEW OF LITERATURE

The first dwarf mutation in chickens, found by Landauer (1929) in Rhode Island Reds, is characterized by subnormal growth in body weight and bone length, enlarged thyroid glands, and a reduced number of bone marrow cells (Landauer, 1929; Upp, 1932; Hutt, 1949). It is usually semi-lethal before the onset of sexual maturity. According to Mayhew and Upp (1932) and Upp (1934), this dwarf is determined by an autosomal recessive gene, for which Hutt (1949) proposed the symbol td. Another dwarf in chickens is a sex-linked recessive mutation, dw, discovered by Hutt (1949). Individuals carrying this gene mature and reproduce essentially normally.

Effects of the Dwarf Gene, dw

In egg production strains Hutt (1953; 1959) found that growth retardation caused by the gene dw was apparent by six weeks of age. At hatching, body weights were equal to those of normal sibs, but at two weeks the dwarfs were 19 percent smaller. Adult body weight was reduced 26 to 32 percent in females and 38 to 46 percent in males. In the latter, bones were 32 percent shorter than in normal sibs. The ratio of egg weight to body weight was about 22 percent greater for dwarfs than for normal hens. Even though the dwarf hens laid larger eggs than normals relative to their body weights,

the total egg mass produced per hen was 21 to 28 percent less for the dwarfs.

Hutt (1959) reported that sexual maturity in pullets was about three weeks later in dwarfs than in normal sisters. The range in days of delay in maturity due to dw was -2 to +34. Hen-day egg production was nine to 14 percent less in dwarfs, while viability, fertility, and hatchability were not affected.

More recently, several workers, including Bernier and Arscott (1966), Arscott and Bernier (1968), Merat (1969), Mohammadian (1969), and Prod'homme and Merat (1969), have presented results similar to those of Hutt (1959). Thus, the reduction in body weight caused by the dwarf gene of about 25 to 35 percent in females and about 30 to 40 percent in males has been confirmed. Shank length is reduced about 25 percent and egg weight seven to 10 percent. The estimated reduction in egg production has ranged from zero to 36 percent, while that for sexual maturity has averaged seven to 14 days later in dwarfs compared with normal sisters. Viability, fertility, and hatchability are apparently not affected by dwarfism (Merat, 1969).

The effects of dw in broiler females was studied by Mohammadian (1969). Body weights were lowered 24 to 37 percent at eight weeks and 21 to 29 percent at 22 weeks. Mature shank length was 18 to 21 percent lower. Egg size

in dwarf broilers was eight to 10 percent lower than in normal sibs. Thus the reduction in growth and egg size seems to be nearly the same in broilers as in egg-type chickens. The above worker found that rate of egg production was lower in dwarf hens from a second backcross (7/8 broiler) of heterozygous males with normal broiler dams, but nearly the same in hens from the first backcross (3/4 broiler). Jaap (1969), Jaap and Mohammadian (1969), and Mohammadian (1969), suggested that the rate of yolk formation might be lower in dwarf than in normal broiler pullets, but the dwarfs produced better shell quality and fewer double yolks than their normal sibs. Ovary weights may be lower in dwarf than in normal hens (van Tienhoven et al, 1966).

Mohammadian (1970) found a 2.8 percent increase in eight-week body weight of daughters and a 2.9 percent decrease in weight of heterozygous sons from a mating of normal broiler sires to dwarf dams compared to progeny of normal sires mated to normal dams. Dwarf pullets produced from dwarf sires mated to dwarf dams were 4.4 percent smaller than dwarf pullets produced from dwarf sires mated to normal dams. Cockerels from a dwarf x dwarf mating were 28.1 percent smaller than from a homozygous normal x dwarf mating.

Nutritional Experiments on Dwarf Females

The efficiency of feed conversion of sex-linked dwarfs has been investigated by several workers. Bernier and Arscott (1960) found that dwarf hens weighing 37 percent less than their normal sisters ate 34 percent less feed. On a per dozen eggs basis, the dwarfs required 26 percent less feed. When egg size was corrected to 24 ounces per dozen eggs, 16 percent less feed was required. In a later study by Bernier and Arscott (1966), body weight of dwarf pullets averaged 25 to 30 percent less than that of normal pullets over a wide range of ages (4, 8, 12, 17, and 23 weeks). The percentage difference in feed consumption between dwarfs and normals was nearly the same as the difference for body weight.

Prod'homme and Merat (1969) tested the feed efficiency of 40 normal and 40 dwarf sisters from a heavy-breed strain. The dwarf hens laid almost the same number of eggs as the normal sibs but feed consumption was 26 percent less. However, this was accounted for by 34 percent less body weight and nine percent less egg weight for the dwarfs. In a comparison of efficiency of egg production of dwarf White Leghorns with normal sisters, Selvarajah et al. (1970) found that dwarf hens required 2.48 units of feed to produce a unit of egg mass while the normal sisters required 2.78 units. On the other hand, Guillaume (1969) found that dwarf

pullets consumed as much feed as normal sisters of the same body weight. Furthermore, because the dwarfs gained weight more slowly, intake of feed per unit gain was greater, and efficiency of protein utilization was lower than for normals. Much of the weight gain in dwarf hens was attributed to an increase in tissue lipid.

Dwarf layers seem to perform better when fed levels of protein greater than 14 percent. Arscott et al. (1961) reported that both normal and dwarf hens fed an 18 percent protein ration consumed less feed than hens fed a 15.7 percent protein ration. The normal hens laid fewer eggs than their dwarf sisters fed the higher protein level. Arscott and Bernier (1968) compared rations containing protein levels of 12 to 21 percent fed to normal and dwarf hens. Dwarf hens fed the highest level of protein laid the largest eggs. In contrast, with normal hens there was no advantage in egg size for rations containing more than 16 percent protein. Egg production and feed per dozen eggs seemed to plateau at 15 percent for dwarfs, but at 14 percent for normals. Feed consumption seemed to vary with the percent protein in the ration for the dwarfs but not for the normal hens. For both dwarfs and normals, body weight varied with the protein level. Overall, the dwarfs weighed 26 percent less, laid 13 percent fewer eggs, and consumed 27 percent less feed than the normal sisters. Similar

results were reported by Quisenberry et al. (1969). Dwarf hens fed protein levels greater than 14 percent had greater egg size, egg number, and feed efficiency. Except for egg numbers this was also observed for normal hens. On the other hand, Magruder and Coune (1969) found that different protein levels, with or without vitamin or energy supplementation, did not appreciably affect either hen-day egg production or feed consumed per dozen eggs, of either dwarf or normal White Leghorns.

Arscott et al. (1961, 1962) reported that high calcium and phosphorus levels (2.3 and 3.0 percent) in the diet of dwarf hens increased egg production more than in normal hens. Furthermore, the specific gravity of eggs was better for all hens, especially dwarfs, on high calcium and phosphorus levels. Prod'homme and Merat (1969), however, failed to detect any effect of different dietary levels of calcium and phosphorus on shell quality, egg number, or egg weight of either normal or dwarf hens.

Physiology of Dwarfism

The effects of dwarf genes on the endocrine system in chickens has been studied by several workers. Upp (1932) treated autosomal dwarfs with anterior pituitary tissue and with sex hormones derived from the pituitary; these treatments had no effect on growth and fertility. The anterior

pituitaries of the dwarfs had normal gonadotrophic and thyrotrophic cells. Thyroid malfunction seems not to be involved in sex-linked dwarfism (van Tienhoven et al., 1966). Treating dwarfs with thyroxine did not alter the manifestation of dwarfism. On the other hand, Merat and Guillaume (1969) concluded that possible hypothyroidism in dwarf chicks was indicated by a reduced size of the thyroid, a greatly increased fat percentage, a lowered rate of thyroid secretion, and a lower metabolic rate. Similarly, Rajaratnam et al. (1969) concluded that low thyroxine secretion may be partly responsible for low body weight and feed intake. Dwarf pullets treated from one day to nine weeks of age with thyroprotein (one percent thyroxine activity) gained significantly more in body weight than the untreated dwarf controls. Weight gains for normal pullets were nearly the same in the treated and untreated groups.

Rajaratnam et al. (1969) and Summers et al. (1970) found significantly lower body temperature and oxygen consumption in dwarfs than in normal layers. Feeding thyroxine or thyroprotein increased temperature to a normal level. In the Summers et al. (1970) study, thyroxine treatment also increased gain in weight.

MATERIALS AND METHODS

Genetic Stocks

Chickens used in this study were White Leghorn lines, B and C, selected over nine generations for large and small body size, respectively (Festing and Nordskog, 1967). Hence, the polygenes for body size in the two lines represent extremes. In addition, a line D, of mixed origin, carried a sex-linked recessive dwarf gene, dw, and polygenes assumed to be intermediate for body size.

Breeding Scheme

From the above lines, two 2x2 diallel sets of crosses were produced in each of two years, 1968 and 1969. The experimental plan was to study the effects of the dwarf gene in contrasting genetic backgrounds of polygenes for extremes in body size on performance of the female offspring. Hens were artificially inseminated with pooled semen from several males. The first diallel set consisted of the progeny from the pure lines B and D and from their reciprocal cross matings. The second diallel set consisted of the corresponding progeny from the dwarf line and the small Leghorn line C. In addition, data gathered in 1967 on the progeny of the reciprocal cross matings only, was included as a preliminary study.

The two diallel sets are represented as follows:

Diallel Set B

Sire line \ Dam line	B	D
B	BB	BD
D	DB	DD

Diallel Set C

Sire line \ Dam line	C	D
C	CC	CD
D	DC	DD

Thus, for example, BD represents the progeny of line B males mated to line D females and DB represents the progeny from the reciprocal cross.

The sex chromosome and autosomal composition of the above progeny is shown in Table 28. Each pullet receives a W sex chromosome from its dam and a Z chromosome from its sire. The W chromosome is assumed to be genetically inert. The dwarf sires transmit a Z chromosome carrying the dwarf gene dw to each of their female progeny. Thus, since the dwarf gene is completely recessive to its normal allele, the genotypes Z_2Z_2 (males) and Z_2W (females) produce dwarfs. In particular, these would be represented by the DB and DC cross pullets and by the pure line D (i.e., DD) progeny. The cross line males, being heterozygous, would be normal. We assume that the Z chromosome, as well as the autosomes, carry polygenes for quantitative traits. Body size, therefore, would be determined by a major dwarf locus and by polygenes. Thus, the BD pullets would receive autosomes from line B

Table 28. Chromosome[a] makeup in the progeny of the dwarf cross matings

			Dam line[b]	
			Leghorn	Dwarf
			1	2
			Z_1W_1 A_1A_1	Z_2W_2 A_2A_2
Female progeny				
Sire line	1	Z_1Z_1 A_1A_1	Z_1W_1 A_1A_1	Z_1W_2 A_1A_2
	2	Z_2Z_2 A_2A_2	Z_2W_1 A_2A_1	Z_2W_2 A_2A_2
Male progeny				
Sire line	1	Z_1Z_1 A_1A_1	Z_1Z_1 A_1A_1	Z_1Z_2 A_1A_2
	2	Z_2Z_2 A_2A_2	Z_2Z_1 A_2A_1	Z_2Z_2 A_2A_2

[a]Chromosomes:

Z_i - sex chromosome Z, i = 1,2

W_i - sex chromosome W, i = 1,2

A_i - set of autosomes, i = 1,2

[b]Line 1 is either large Leghorn line B or small Leghorn line C; line 2 is the dwarf line D.

sires carrying polygenes for high body weight and autosomes from line D dams carrying polygenes for intermediate body size. The reciprocal cross pullets, DB, would carry, in addition, the major recessive gene for dwarfism. In contrast, the crosses of line D with line C would carry equal doses of polygenes for intermediate and small body size.

The offspring from the matings of line B are designated the B diallel set and those from line C the C diallel set, even though the matings were not truly diallelic. That is, separate pens (different matings) were used to produce either pure lines or crosses. For convenience, progeny of matings within lines are called "pure" lines to contrast then with line cross progeny. Finally, the same group of pure line D chickens were used in both diallel sets B and C.

Management of Stocks

Difficulty with fertility in 1968 was encountered because some matings produced few or no offspring in some hatches. A total of six hatches was obtained. In 1969, sufficient progeny from all matings were produced in two hatches.

In 1968 all offspring were brooded and reared together to 16 weeks of age. The chickens were then sorted into two size groups and placed in separate pens to eliminate competition due to size differences. All males were discarded except for a few pure line males saved after measurements

were taken at 22 weeks. Because the number of birds was small, the fourth hatch was discarded at 22 weeks. From the remaining hatches, fifteen pullets per line or cross and per hatch were placed on separate slat floor pens for the egg production test. Pens were assigned to lines or crosses at random within hatches. Pure line C pullets (CC), available in all hatches, served as controls.

In 1969 the C diallel set was brooded and reared separately from the B diallel set. Twenty-five pullets per hatch of each mating type (e.g., B x B, B x D) were randomly assigned to cages at 19 weeks of age.

Traits Studied

Measurements were taken on body weight, egg weight, shank length, sexual maturity, egg production, fertility, and hatchability (Table 29). The early body weights (1, 10, and 21 days) in 1969 were taken only in the second hatch. Individual egg production was recorded on four consecutive days per week from 22 to 26 weeks of age in 1968 and for the entire laying period from 19 to 50 weeks in 1969. Pen records were kept for the remainder of the production tests (27 to 50 weeks) in 1968. Sexual maturity (age at first egg) was recorded within the production period from 22 to 26 weeks of age in 1968 and within the period from 19 to 32 weeks of age in 1969.

Feed efficiency was also measured in 1969. Feed re-

Table 29. Traits measured in each of the three years of the study (indicated by X)

Sex	Trait	Age	Year 1967	1968	1969
Males	Body wt.	1 day			X
		10 days			X
		21 days			X
		8 wk	X	X	X
		22 wk	X	X	X
	Shank length	8 wk	X		
		22 wk	X	X	X
	Fert. & hatch.			X	X
Females	Body wt.	1 day			X
		10 days			X
		21 days			X
		8 wk	X	X	X
		22 wk	X	X	X
		32 wk		X	X
		50 wk			X
	Shank length	8 wk	X		
		22 wk	X	X	X
	Egg wt.	32 wk		X	X
		50 wk		X	X
	Sex. mat.	22-26 wk		X	
	(first egg)	19-32 wk			X
	% prod. (hen-day)			X	X
	Fert. & hatch.			X	X
	Feed efficiency	30-46 wk			X

quired for body weight maintenance and for producing eggs was estimated on 15 hens for each hatch and line or cross, except line D, during three five-week periods (September through December). Egg and body weights were taken at the beginning and end of each period and egg production was recorded daily. The hens were individually fed a measured quantity of feed of a standard ration. For the feed allotment a special can of known capacity was used. The variation in the weight of the can filled with feed was determined from 328 separate weighings over the 15 weeks of the experiment.

Statistical Procedure

General model

The analysis of the data was based on the model

$Y_{ijklm} = u + A_i + A_j + Z_i + H_{ij} + M_j + E_{ijkl} + e_{ijklm}$, where

u = general mean

A_i = effect of the autosomes from sire line i, i = 1,2

A_j = effect of the autosomes from dam line j, j = 1,2

Z_i = effect of the sex chromosome from sire line i

H_{ij} = heterosis effect of sire line i mated with dam line j

M_j = maternal egg size effect of dam line j

E_{ijkl} = effect of the l^{th} hatch within the k^{th} year on the progeny of the i x j mating

e_{ijklm} = effect of the m^{th} individual of the l^{th} hatch and k^{th} year from the mating i x j.

The mean of t individuals per hatch in s hatches is,

$$Y_{ij\cdots} = u + A_i + A_j + Z_i + H_{ij} + M_j + \sum_{l=1}^{s} E_{ijkl}/s + \sum_{l=1}^{s}\sum_{m=1}^{t} e_{ijklm}/st.$$

Thus, for a single hatch the residual error is $E_{ijkl} + \sum_{m=1}^{t} e_{ijklm}/t$. It was assumed that, $\Sigma A_i = \Sigma A_j = \Sigma H_{ij} = \Sigma M_j = \Sigma E_{ijkl} = \Sigma e_{ijklm} = 0$, $H_{ij} = H_{ji}$, and that, for the crosses, each line contributed one half of the autosomal effects. The W sex chromosome was assumed to be genetically inactive and, hence, was ignored. The maternal effect was assumed to be due entirely to the effect of different egg sizes of the dam parents; therefore, the terms "maternal effect" and "egg size effect" are used synonomously in Part II of this thesis.

Means

The means for all traits were calculated within and across hatches. The means were then averaged across years and summarized for each trait in a single 2x2 diallel set (Table 30). The effects represented by the general model were then estimated from the means of each 2x2 diallel set.

Estimation of effects

The effects we wish to estimate are,

Autosomal: $a = 1/2(A_1 - A_2)$

Sex chromosome: $z = 1/2(Z_1 - Z_2)$

Table 30. Mean values in the diallel set[a]

	Dam line	1	2	
Sire line	1	$\hat{Y}_{11\cdot}$	$\hat{Y}_{12\cdot}$	$\hat{Y}_{1\cdot\cdot}$
	2	$\hat{Y}_{21\cdot}$	$\hat{Y}_{22\cdot}$	$\hat{Y}_{2\cdot\cdot}$
		$\hat{Y}_{\cdot 1\cdot}$	$\hat{Y}_{\cdot 2\cdot}$	$\hat{Y}_{\cdot\cdot\cdot}$

[a]Line 1 is either Leghorn line B or Leghorn line C; line 2 is D.

Heterosis: $h = 1/2[(H_{12} + H_{21})/2 - (H_{11} + H_{22})/2]$

Maternal: $m = 1/2(M_1 - M_2)$.

In a 2x2 diallel set, since there are only three degrees of freedom, only three effects can be independently estimated. In female progeny a, h, and z are estimable but m is not; in male progeny h and z are estimable, while a and z are confounded so that only the joint effect is estimable.

Estimated values will be designated with a hat (^), such as $\hat{a}$ and $\hat{Y}_{ij}$, to contrast them with the corresponding expected values, a and Y_{ij}.

Males The general model was modified slightly to account for the fact that males carry two sex chromosomes. Also, since the effects were estimated from a single 2x2

table of means for each trait, no error estimate was obtained. Hence, the error terms (E + e) were deleted from the model. The equation for males is,

$$Y_{ij\cdot} = u + A_i + A_j + Z_i + Z_j + H_{ij} + M_j.$$

The matrix of coefficients for this model is given in Table 31 and the equations are

$$Y_{11\cdot} = u + A_1 + Z_1 + H_{11} + M_1$$

$$Y_{12\cdot} = u + 1/2\ A_1 + 1/2\ A_2 + 1/2\ Z_1 + 1/2\ Z_2 + H_{12} + M_2$$

$$Y_{21\cdot} = u + 1/2\ A_1 + 1/2\ A_2 + 1/2\ Z_1 + 1/2\ Z_2 + H_{21} + M_1$$

$$Y_{22\cdot} = u + A_2 + Z_2 + H_{22} + M_2.$$

The estimate of sire effects is (Table 30),

$$(\hat{Y}_{11\cdot} + \hat{Y}_{12\cdot})/2 - (\hat{Y}_{21\cdot} + \hat{Y}_{22\cdot})/2 = (\hat{Y}_{1\cdot\cdot} - \hat{Y}_{2\cdot\cdot})$$

which can be set equal to,

$$1/2(A_1 - A_2) + 1/2(Z_1 - Z_2) = a + z.$$

The estimate of the dam effects is,

$$(\hat{Y}_{11\cdot} + \hat{Y}_{21\cdot})/2 - (\hat{Y}_{12\cdot} + \hat{Y}_{22})/2 = (\hat{Y}_{\cdot 1\cdot} - \hat{Y}_{\cdot 2\cdot})$$

which we set equal to,

$$1/2(A_1 - A_2) + 1/2(Z_1 - Z_2) + (M_1 - M_2) = a + z + 2m.$$

Finally, the estimate of the maternal effect is,

$$\hat{m} = [(\hat{Y}_{\cdot 1\cdot} - \hat{Y}_{\cdot 2\cdot}) - (\hat{Y}_{1\cdot\cdot} - \hat{Y}_{2\cdot\cdot})]/2.$$

The estimate of heterosis is one-half of the mean of the crosses minus the mean of the pure lines. It measures the amount of interaction between sire lines and dam lines:

$$\hat{h} = 1/2[(\hat{Y}_{12\cdot} + \hat{Y}_{21\cdot})/2 - (\hat{Y}_{11}/ + \hat{Y}_{22\cdot})/2]$$

Table 31. Matrix of coefficients for estimating effects

Sex	Mean	Lines and crosses	u	A_1	A_2	Z_1	Z_2	H	M_1	M_2
Males	Y_{11}·	BB,CC	1	1	0	1	0	1	1	0
	Y_{12}·	BD,CD	1	1/2	1/2	1/2	1/2	1	0	1
	Y_{21}·	DB,DC	1	1/2	1/2	1/2	1/2	1	1	0
	Y_{22}·	DD	1	0	1	0	1	1	0	1
Females	Y_{11}·	BB,CC	1	1	0	1	0	1	1	0
	Y_{12}·	BD,CD	1	1/2	1/2	1	0	1	0	1
	Y_{21}·	DB,DC	1	1/2	1/2	0	1	1	1	0
	Y_{22}·	DD	1	0	1	0	1	1	0	1

which can be set equal to,

$$1/2\ [(u + 1/2\ A_1 + 1/2\ A_2 + 1/2\ Z_1 + 1/2\ Z_2 + H_{12} + M_2) + (u + 1/2\ A_1 + 1/2\ A_2 + 1/2\ Z_1 + 1/2\ Z_2 + H_{21} + M_1)]/2$$
$$- [(u + A_1 + Z_1 + H_{11} + M_1) + (u + A_2 + Z_2 + H_{22} + M_2)]/2$$
$$= 1/2\ [(H_{12} + H_{21})/2 - (H_{11} + H_{22})/2].$$

This is essentially the classical definition of heterosis, except that $(\hat{Y}_{11\cdot} + \hat{Y}_{22\cdot})/2$ is not strictly the true midparent mean but rather the mean of the midparental breed types from the same generation as the F_1 crosses. In fact, all of the Y_{ijk}'s were from the same generation.

Females The general model applies except that, as with males, the errors (E + e) were not estimated. The equations for females, obtained from the matrix of coefficients in Table 31, are

$$Y_{11\cdot} = u + A_1 + Z_1 + H_{11} + M_1$$
$$Y_{12\cdot} = u + 1/2\ A_1 + 1/2\ A_2 + Z_1 + H_{12} + M_2$$
$$Y_{21\cdot} = u + 1/2\ A_1 + 1/2\ A_2 + Z_2 + H_{21} + M_1$$
$$Y_{22\cdot} = u + A_2 + Z_2 + H_{22} + M_2.$$

The maternal egg size effect (m) and autosomal effects (a) are confounded in females:

$$(Y_{11\cdot} + Y_{21\cdot})/2 - (Y_{12\cdot} + Y_{22\cdot})/2$$
$$= (2u + 3/2A_1 + 1/2\ A_2 + Z_1 + Z_2 + H_{11} + H_{21} + 2M_1)/2$$
$$- (2u - 1/2\ A_1 + 3/2A_2 + Z_1 + Z_2 + H_{12} + H_{22} + 2M_2)/2,$$

$= 1/2(A_1 - A_2) + (M_1 - M_2) = a + 2m$, assuming $H_{12} = H_{21}$ and $H_{11} = H_{22}$.

Except for an adjustment for egg size effect on body weight at eight weeks in all three years and at 1, 10, and 21 days in 1969 in the B diallel set females (described in the Results section), m was assumed to be zero. Hence, from Table 30,

$$\hat{a} = (\hat{Y}_{11\cdot} + \hat{Y}_{21\cdot})/2 - (\hat{Y}_{12\cdot} + \hat{Y}_{22\cdot})/2$$
$$= (\hat{Y}_{\cdot 1\cdot} - \hat{Y}_{\cdot 2\cdot}).$$

The average sex chromosome effect, $z = 1/2(Z_1 - Z_2)$, was estimated from

$$2\hat{z} = [(\hat{Y}_{11\cdot} + \hat{Y}_{12\cdot})/2 - (\hat{Y}_{21\cdot} + \hat{Y}_{22})/2]$$
$$- [(\hat{Y}_{11\cdot} + \hat{Y}_{21\cdot})/2 - \hat{Y}_{12\cdot} + \hat{Y}_{22\cdot})/2]$$
$$= (\hat{Y}_{1\cdot\cdot} - \hat{Y}_{2\cdot\cdot}) - (\hat{Y}_{\cdot 1\cdot} - \hat{Y}_{\cdot 2\cdot}).$$

This is equivalent to taking the difference between the means of the reciprocal crosses. The expected value is,

$$[(u + 3/4A_1 + 1/4\ A_2 + Z_1 + 1/2\ H_{11} + 1/2\ H_{12} + 1/2\ M_1 +$$
$$1/2\ M_2)$$
$$- (u + 1/4\ A_1 + 3/4A_2 + Z_2 + 1/2\ H_{21} + 1/2\ H_{22} +$$
$$1/2\ M_1 + 1/2\ M_2)]$$
$$- [(u + 3/4A_1 + 1/4\ A_2 + 1/2\ Z_1 + 1/2\ Z_2 + 1/2\ H_{11} +$$
$$1/2\ H_{21} + M_1)$$
$$- (u + 1/4\ A_1 + 3/4\ A_2 + 1/2\ Z_1 + 1/2\ Z_2 + 1/2\ H_{12} +$$
$$1/2\ H_{22} + M_2)]$$
$$= 1/2\ (A_1 - A_2) + (Z_1 - Z_2) - 1/2\ (A_1 - A_2)$$

$= (Z_1 - Z_2)$, assuming a zero maternal effect.

The $\hat{z}$ estimate can be compared with the estimated effects of the autosomes ($\hat{a}$) and heterosis ($\hat{h}$) to show the relative importance of each.

Because the sex chromosome effect was assumed to be due entirely to the dwarf gene, the $\hat{dw}$ effects presented in the results as dwarf gene effects are equivalent to sex chromosome effects, $\hat{z}$. To demonstrate the reduction in traits due to the dwarf gene, a relative dwarf effect is defined in percent as the difference between the means of the reciprocal crosses divided by the mean of the larger cross, or

$$\hat{dw} = [(\hat{Y}_{12\cdot} - \hat{Y}_{21\cdot})/\hat{Y}_{12\cdot}] \ (100).$$

Heterosis effects in females would be estimated in the same way as in males,

$$\hat{h} = 1/2 \ [(\hat{Y}_{12\cdot} + \hat{Y}_{21\cdot})/2 - (\hat{Y}_{11\cdot} + \hat{Y}_{22\cdot})/2].$$

Analysis of variance

The form of the analysis of variance of each trait for each diallel set, assuming years random and sire and dam lines fixed, is

Source	d. f.	Expected mean squares
Years	1	$\sigma_e^2 + 4\sigma_y^2$
Sire lines (S)	1	$\sigma_e^2 + 4K_s^2$
Dam lines (D)	1	$\sigma_e^2 + 4K_d^2$
S x D	1	$\sigma_e^2 + 2K_{sd}^2$
Error	3	σ_e^2

This corresponds to the general model except that the effects are in terms of sires, dams, and replications,

$$Y_{ijklm} = u + S_i + D_j + (SD)_{ij} + R_k + \sum_{l=1}^{s} E_{ijkl/s} + \sum_{l=1}^{s}\sum_{m=1}^{t} e_{ijklm/st},$$

where,

u = general mean

S_i = sire line effect, $i = 1,2$

D_j = dam line effect, $j = 1,2$

$(SD)_{ij}$ = interaction caused by mating sire line i with dam line j

R_k = effect of year replications, $k = 1,2$

E_{ijkl} = effect of the l^{th} hatch within the k^{th} year on progeny from the i x j mating

e_{ijklm} = effect of the m^{th} progeny of the l^{th} hatch and the k^{th} year from the mating i x j

s = number of hatches

t = number of individuals per hatch.

The expected value of the sire line variance component, κ_s^2 is $(a + 2z)^2$, while that for the dam line component, κ_d^2, is $(a + 2m)^2$. Thus, if the maternal effect m equals zero, the importance of the sex chromosomes (i.e., in this case, the dwarf gene) in the female progeny can be estimated by comparing the expected mean square for sire lines with that for dam lines. Also, since the interaction of the sire line with the dam line is assumed to be due to heterosis effect (h),

the expected value of the interaction variance, $\mathcal{K}^2_{Sd}$, is h^2.

Feed efficiency experiment

Efficiency of egg production was evaluated in three ways. The first was the ratio of average egg mass produced per day ($\bar{M}$) divided by the average daily feed consumption ($\bar{F}$). In the second, a multiple regression equation was used. Average daily feed consumption (F) was regressed on initial body weight (W), on change in body weight (ΔW), and on average egg mass produced per day (M). The equation is

$$F = \hat{a} + \hat{b}_W W + \hat{b}_{\Delta W} \Delta W + \hat{b}_M M + e,$$

where $\hat{a}$ is the slope intercept feed constant, $\hat{b}_W$ is the estimated feed required per day to maintain a unit of body weight, $\hat{b}_{\Delta W}$ is the estimated feed required for a unit change in body weight, and $\hat{b}_M$ is the estimated feed required per day per unit of egg mass produced. Efficiency for a genetic group with mean body weight $\bar{W}$ and mean egg mass $\bar{M}$ was then estimated as

$$\frac{\hat{b}_M \bar{M}}{\hat{b}_M \bar{M} + \hat{b}_W \bar{W}}.$$

Finally, efficiency was estimated indirectly from the ratio $\bar{M} / \bar{W}$ for each genetic group.

RESULTS

Males

Body weights of cockerels at 1, 10, and 21 days of age from hatch 2, 1969, are given in Table 32. The BB and DB chicks, both from line B dams, weighed 46 and 44 grams, respectively. Chicks from the dwarf-line dams weighed about 29 grams in both the B and C groups. Thus, chicks from the same dam with or without the dwarf gene weighed nearly the same at hatching. The large egg size effect persisted at least through 21 days. Since egg size was nearly the same for lines C and D, the difference in the maternal effect or egg size effect was essentially zero.

The means of body weight and shank length for males at eight and 22 weeks are presented in Tables 33 through 36. From these the effects $\hat{h}$ and $\hat{m}$ were estimated (Tables 37 and 38). An egg size effect on body weight persisted to eight weeks in the B diallel set ($\hat{m}$ = 55.2, Table 37). The estimate at 22 weeks was small, although the DB cockerels were consistently larger than the reciprocal cross cockerels BD over the three years. The effect $\hat{m}$ was essentially zero in all cases in Table 38. As expected, some heterosis was evident in the crosses of both the B and C diallel sets.

The body weights of the B cross pullets at 1, 10, and 21 days of age in hatch 2, 1969, and at eight weeks in all three years were adjusted for m by extrapolating from the

Table 32. Body weights (gm) of young cockerels from hatch 2, 1969

Diallel set B					Diallel set C				
Lines and crosses	No.	Age (days) 1	10	21	Lines and crosses	No.	Age (days) 1	10	21
BB	17	46	102	233	CC	17	29	61	137
BD	15	29	83	196	CD	13	31	72	154
DB	15	44	103	234	DC	15	30	75	155
DD	20	28	53	119	DD	20	28	53	119

Table 33. Body weights (gm) of males at eight and 22 weeks in the B diallel set

Age (wk)	Lines and crosses	1967	1968	1969	Av.	No.
8	BB		1173	1002	1088	129
	BD	785	997	894	892	72
	DB	901	1104	1002	1003	126
	DD		471	400	436	53
22	BB		3096	3292	3194	41
	BD	2411	2520	2583	2505	41
	DB	2511	2660	2665	2612	62
	DD		1185	858	1022	42

Table 34. Shank lengths (cm) of males at eight and 22 weeks in the B diallel set

Age (wk)	Lines and crosses	1967	1968	1969	Av.	No.
8	BB					
	BD	6.7				17
	DB	7.0				25
	DD					
22	BB		10.5	11.6	11.1	41
	BD	9.9	10.0	10.9	10.3	41
	DB	10.1	10.0	10.6	10.2	62
	DD		6.3	6.4	6.4	42

Table 35. Body weights (gm) of males at eight and 22 weeks in the C diallel set

Age (wk)	Lines and crosses	1967	1968	1969	Av.	No.
8	CC		612	588	600	177
	CD	632	714	679	675	83
	DC	600	733	684	672	152
	DD		472	400	436	53
22	CC		1385	1278	1330	64
	CD	1471	1625	1666	1588	37
	DC	1516	1612	1630	1586	85
	DD		1185	858	1022	41

Table 36. Shank lengths (cm) of males at eight and 22 weeks in the C diallel set

Age (wk)	Lines and crosses	1967	1968	1969	Av.	No.
8	CC					
	CD	6.3				12
	DC	6.2				40
	DD					
22	CC		7.8	8.9	8.4	64
	CD	8.8	8.8	9.7	9.1	37
	DC	8.7	8.8	9.5	9.0	85
	DD		6.3	6.4	6.4	41

male data (Table 37). For example, in 1969, at one day of age, m was equal to 7.5 in the cockerels. This was added to the BD and DD pullet one-day weights and subtracted from the BB and DB pullet weights. All corrections were made within years.

Analyses of variance for body weights of cockerels at eight and at 22 weeks are given in Table 39. The relatively high contribution of the dam component to the variance in the B diallel set at eight weeks reflects the influence of egg size on body size. The egg size effect was much less at eight than at 22 weeks as shown by the nearly equal dam and sire line components at the latter age.

Females

Early body weights of pullets in the B diallel set from hatch 2, 1969, are given in Table 40. The dwarf females in this and subsequent tables and figures will be indicated by an asterisk (e.g., *DB). As in males, body weights of the BB and *DB pullets were nearly equal because of the common egg size effect persisting through 21 days of age. All body weights at one day of age in the B diallel set were nearly equal after adjusting for m (Table 41). No egg size effect was detected in diallel set C. The effect of the dwarf gene on chick weight was clearly evident for both diallel sets by 10 days of age.

Table 37. Estimates of maternal ($\hat{m}$) and heterosis ($\hat{h}$) effects in males of the B diallel set

Trait	Age	$\hat{m}$ 1967	$\hat{m}$ 1968	$\hat{m}$ 1969	$\hat{m}$ Av.	$\hat{h}$ 1968	$\hat{h}$ 1969	$\hat{h}$ Av.
Body wt. (gm)	1 day			7.5			0.0	
	10 days			10.1			7.7	
	21 days			18.9			19.9	
	8 wk	57.9	53.9	53.7	55.2	89.3	123.5	106.4
	22 wk	49.9	70.3	40.8	53.7	225.0	274.7	249.8
Shank length (cm)	8 wk	0.2						
	22 wk	0.1	0.0	-0.2	0.0	0.8	0.8	0.8

Table 38. Estimates of maternal effects ($\hat{m}$) and heterosis ($\hat{h}$) in males of the C diallel set

Trait	Age	$\hat{m}$ 1967	$\hat{m}$ 1968	$\hat{m}$ 1969	$\hat{m}$ Av.	$\hat{h}$ 1968	$\hat{h}$ 1969	$\hat{h}$ Av.
Body wt. (gm)	1 day			-0.4			0.8	
	10 days			1.2			8.3	
	21 days			0.4			13.3	
	8 wk	-16.2	9.2	3.1	-1.3	90.8	93.7	92.2
	22 wk	22.7	-7.8	-18.2	-1.1	166.9	290.6	228.7
Shank length (cm)	8 wk	-0.1						
	22 wk	-0.1	0.0	-0.1	-0.1	0.8	1.0	0.9

Table 39. Analyses of variance of body weights at different ages in males

Diallel set	Age	Source	d.f.	Mean square
B	8 wk	Years	1	25066.68*
		Sire lines (S)	1	148130.10**
		Dam lines (D)	1	288307.12**
		S x D	1	113183.92**
		Error	3	877.72
	22 wk	(Years)		0.00
		Sire lines	1	10.31**
		Dam lines	1	12.65**
		S x D	1	2.42*
		Error	4	0.12
C	8 wk	Years	1	4033.59*
		Sire lines	1	11639.64**
		Dam lines	1	15577.59**
		S x D	1	68012.85**
		Error	3	208.82
	22 wk	Years	1	0.086
		Sire lines	1	0.270
		Dam lines	1	0.195
		S x D	1	2.030*
		Error	3	0.069

**P < .01.
*P < .05.

Table 40. Body weights (gm) of pullets at one, 10, and 21 days in the B diallel set not corrected for m (data from hatch 2, 1969)

Lines and crosses	Age (days)		
	1	10	21
BB	45	93	198
BD	29	78	173
*DB	43	90	186
*DD	29	52	117

*Carry the dwarf gene.

The mean body weights at eight, 22, 32, and 50 weeks of age for the B diallel set listed in Table 42 show that the dwarf gene reduced body weight by 25 to 35 percent. The trend observed between ages, although not clear, seemed to show a small decrease in the dwarf effect with age. The dwarf effect was evidently slightly greater in the C diallel set than in the B set (Table 43) and seemed to have slightly less effect on shank length than on body weight (Table 44).

The growth curves at the early ages for pullets from hatch 2, 1969, adjusted for the egg size effect m, are shown in Figure 13. The *DC pullets, even though they carried the dwarf gene, grew faster than the CC pullets. Figure 13 also demonstrates that the dwarf gene exerts its influence soon after the chicks hatch. Growth of the non-dwarf BD pullets slowed considerably at about 20 weeks compared to the BB pullets due to autosome effects and

Table 41. Body weights (gm) and the estimated dwarf effect[a] in pullets at one, 10, and 21 days corrected for m (data from hatch 2, 1969)

Diallel set B					Diallel set C				
Lines and crosses	No.	Age (days) 1	10	21	Lines and crosses	No.	Age (days) 1	10	21
BB	37	37	83	179	CC	37	29	57	115
DB	38	36	87	191	CD	12	30	70	134
*DB	22	36	79	167	*DC	39	28	61	130
*DD	14	36	62	135	*DD	14	28	52	117
[a]Dwarf effect (%)			-9.6	-12.4				-12.4	-3.2

[a]Reduction in body weight of the cross carrying the dwarf gene. For example, in the B diallel set,

$$\frac{(79-87)(100)}{87} = -9.6\%.$$

*Carry the dwarf gene.

Table 42. Body weights (gm) of females at different ages in diallel set B

Age (wk)	Lines and crosses	N	1967	1968	1969	Av.	Dwarf effect[a] (%)
8	BB	148		951	802	876	
	BD	119	782	922	791	832	
	*DB	149	477	594	536	535	-35.6
	*DD	67		491	405	448	
22	BB	132		2370	2388	2379	
	BD	118	1989	2002	2084	2025	
	*DB	148	1421	1371	1476	1423	-29.7
	*DD	61		1003	754	878	
32	BB	108		3007	2904	2956	
	BD	80		2177	2285	2231	
	*DB	94		1726	1645	1686	-24.5
	*DD	57		1120	896	1008	
50	BB	46			3387		
	BD	50			2629		
	*DB	49			1916		-27.1
	*DD	19			958		

[a]Reduction in body weight of the cross carrying the dwarf gene.

*Carry the dwarf gene.

Table 43. Body weights (gm) of females at different ages in diallel set C

Age (wk)	Lines and crosses	N	1967	1968	1969	Av.	Dwarf effect[a] (%)
8	CC	279		485	451	468	
	CD	89	544	606	567	572	
	*DC	226	324	415	393	377	-34.1
	*DD	67		437	351	394	
22	CC	198		1026	1017	1022	
	CD	81	1335	1262	1435	1348	
	*DC	219	695	826	781	767	-43.1
	*DD	61		1003	754	878	
32	CC	173		1081	1075	1078	
	CD	50		1423	1466	1445	
	*DC	101		963	932	948	-34.4
	*DD	57		1120	896	1008	
50	CC	46			1199		
	CD	26			1607		
	*DC	45			1031		-35.9
	*DD	19			958		

[a]Reduction in body weight of the cross carrying the dwarf gene.

*Carry the dwarf gene.

Table 44. Shank lengths (cm) in females at 22 weeks

Lines and crosses	1967	1968	1969	Av.	Dwarf effect[a] (%)
BB		8.5	9.2	8.9	
BD	8.1	8.1	8.9	8.4	
*DB	6.1	6.1	6.3	6.1	-27.4
*DD		5.3	5.6	5.5	
CC		6.5	7.2	6.9	
CD	7.3	7.2	8.2	7.6	
*DC	4.9	5.1	5.6	5.2	-31.6

[a]Reduction in shank length of the cross carrying the dwarf gene.

*Carry the dwarf gene.

presumably to higher egg production of the former (Figure 14). As expected, there was a large difference in growth between the large Leghorn line crosses of diallel set B compared to the small Leghorn line crosses of diallel set C.

The relative reduction in egg weight due to the dwarf gene was greater in the small line C than in the large line B (Table 45). In the former, the decrease was about 11 percent while in the latter it was from 4.4 to 7.5 percent.

Figure 15a shows that the relationship between egg weight and shank length based on the equation $EW = aS^b$ (made linear by plotting on a log-log scale) seems to be different in dwarf compared to normal pullets. Because there were

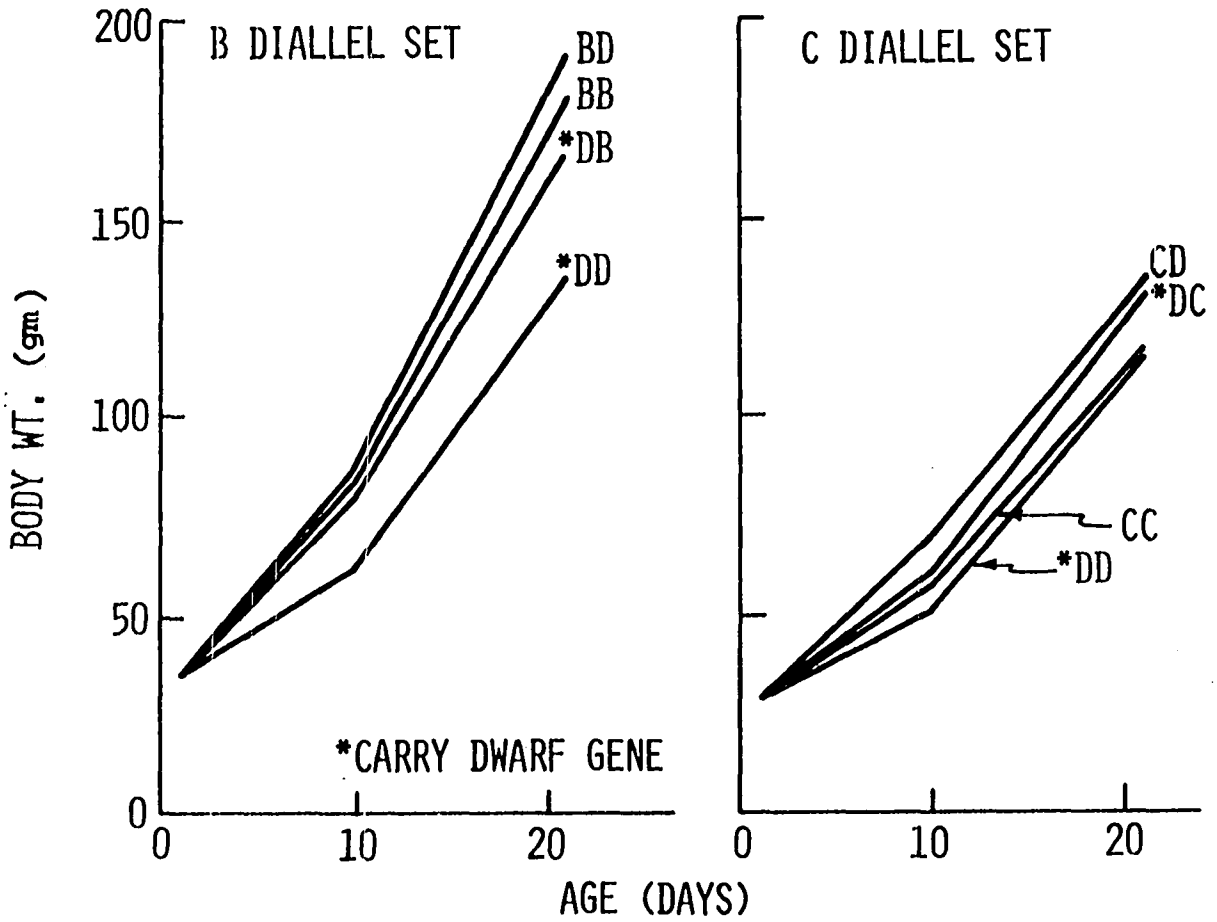


Figure 13. Early growth curves for pullets from hatch 2, 1969.

Figure 14. Growth curves for females (data from both hatches in 1969).

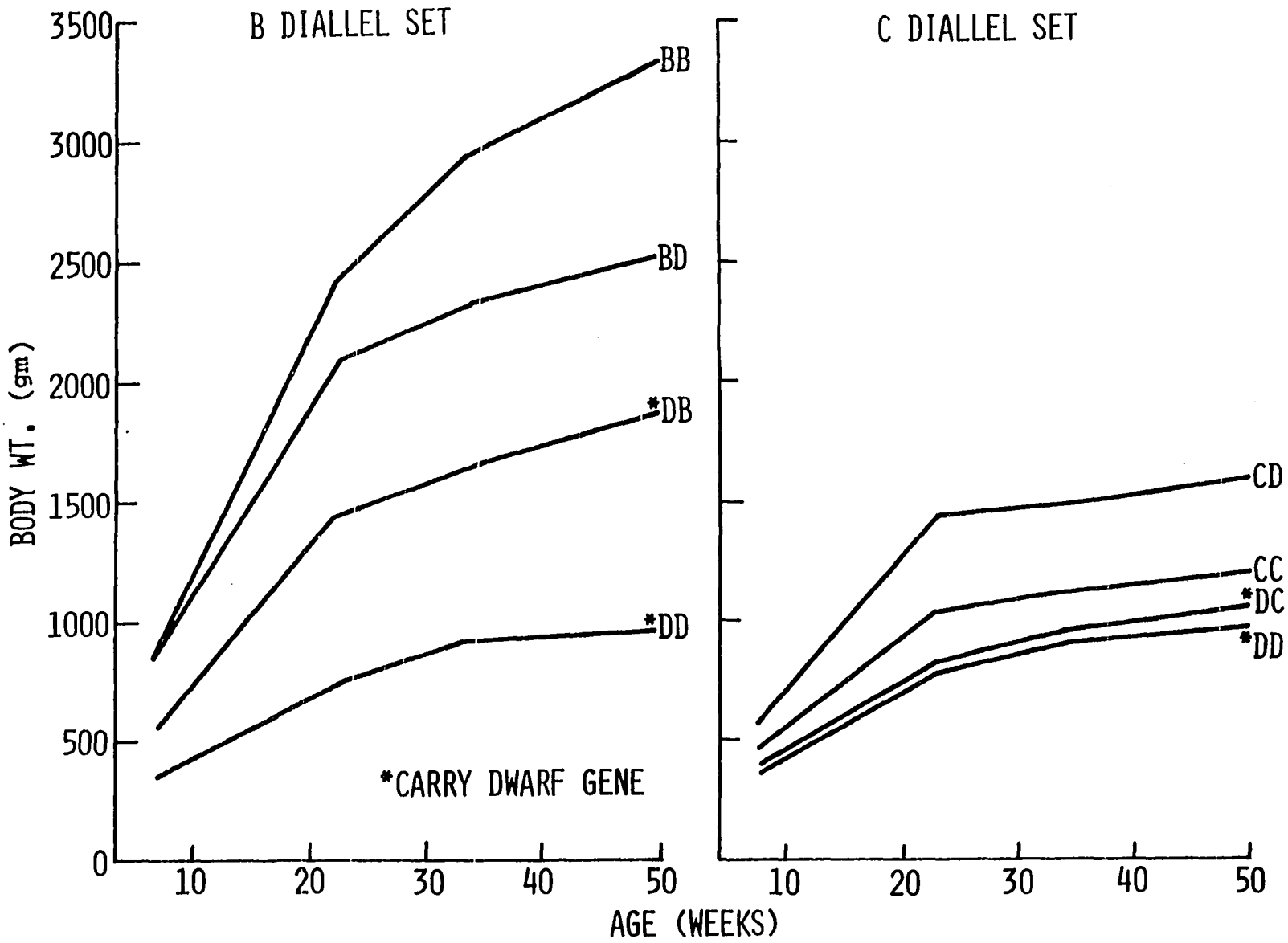

B DIALLEL SET
C DIALLEL SET
BB
BD
*DB
*DD
CD
CC
*DC
*DD
*CARRY DWARF GENE
BODY WT. (gm)
3500
3000
2500
2000
1500
1000
500
0
10
20
30
40
50
AGE (WEEKS)

Table 45. Egg weights (gm) at 32 and 50 weeks of age

Age (wk)	Lines and crosses	1968	1969	Av.	Dwarf effect[a] (%)
32	BB	58.0	58.1	58.0	
	BD	53.1	53.7	53.4	
	*DB	48.9	49.8	49.4	-7.5
	*DD	39.7	37.9	38.8	
	CC	39.0	41.0	40.0	
	CD	42.2	46.4	44.3	
	*DC	38.3	39.0	38.7	-12.6
50	BB	70.0	67.1	68.5	
	BD	59.4	60.0	59.7	
	*DB	57.1	57.1	57.1	-4.4
	*DD	43.1	42.9	43.0	
	CC	44.1	47.5	45.8	
	CD	49.2	51.7	50.5	
	*DC	44.8	45.5	45.1	-10.7

[a]Reduction in egg weight of the cross carrying the dwarf gene.

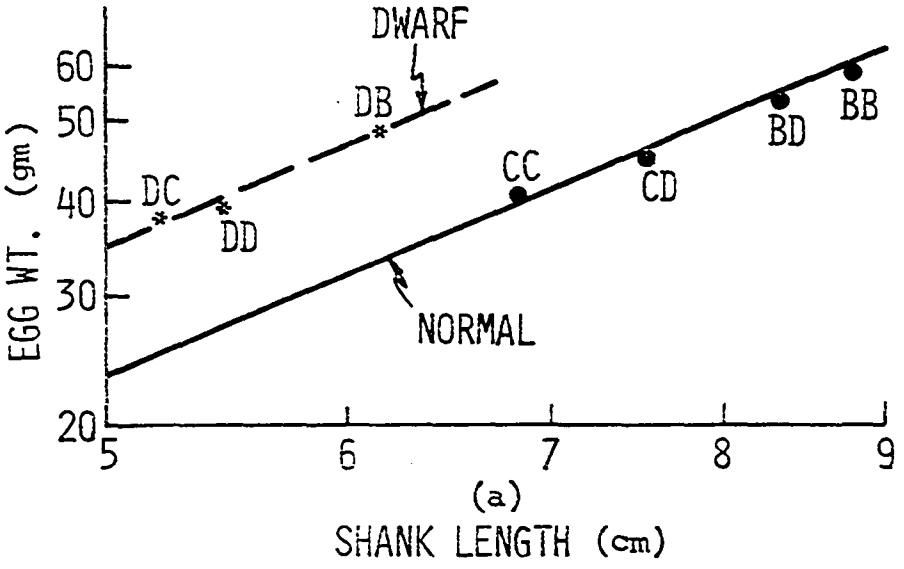
*Carry the dwarf gene.

only three observed points, the slope was only approximated by an eye fit for the dwarfs. The estimated intercepts of the egg weight axis differed by about 33 percent between dwarfs and normals. Thus, the dwarfs laid larger eggs than the normals relative to their shank lengths. Similarly, the dwarf body weights were higher than those for normals relative to shank length (Figure 15b). The estimated intercept was 43 percent higher for the dwarf compared to the non-dwarf females.

The variation in sexual maturity between years (Table 46) was due, in part, to the different time intervals used for estimating maturity. In 1968, the hens were trapnested four days per week for four weeks; in 1969, maturity records were taken over 13 weeks. Also, in 1969 the smaller pullets (*DC and *DD) seemed not to adjust readily to cages. This may have delayed the normal age at first egg. Nevertheless, the results indicate that the dwarf gene delayed sexual maturity by about one week.

Average rate of lay was higher in 1969 than in 1968 (Table 47). This may reflect a management effect: the 1969 pullets were kept in cages while the 1968 pullets were kept in floor pens. The dwarf gene seemed to have only a small effect, if any, on rate of production: the cross-line dwarf hens (*DB, *DC) were slightly poorer layers than the reciprocal cross hens. The reciprocal cross differences for

Figure 15. Log-log plot of relationship between 32-week egg weight and 22-week shank length (a) and between 22-week body weight and shank length (b). The equation for normal pullets was fitted to data from single-trait selection lines Casey (1970).


DWARF
DB
DC
DD
CC
CD
BD
BB
NORMAL
EGG WT. (gm)
60
50
40
30
20
5
6
7
8
9
(a)
SHANK LENGTH (cm)

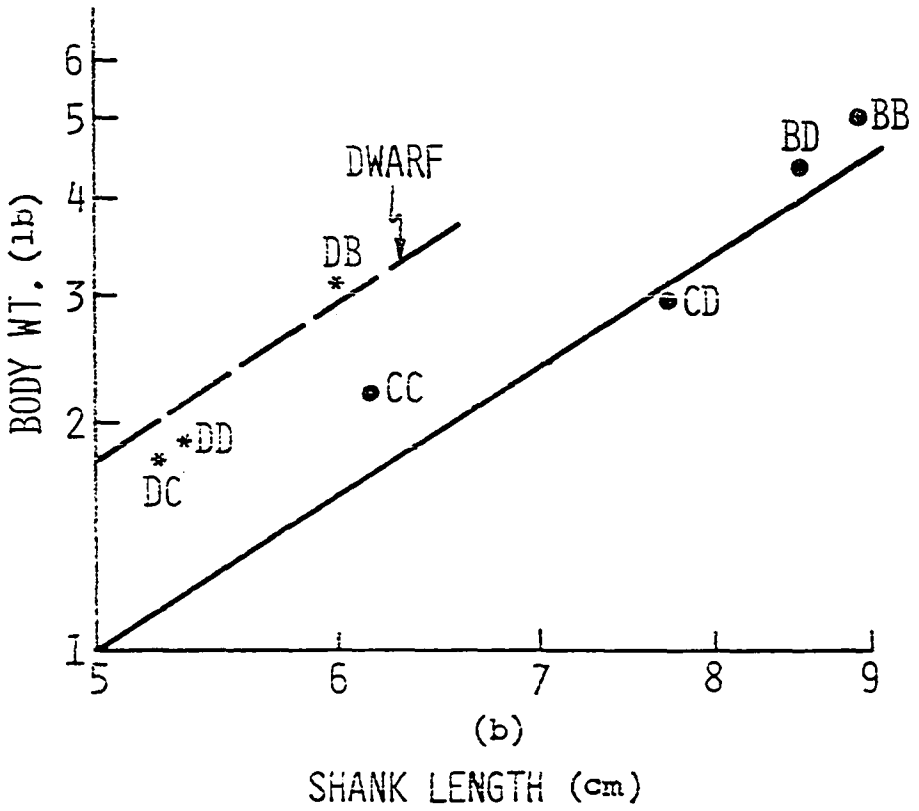

BODY WT. (lb)
6
5
4
3
2
1
DWARF
DB
DC
DD
CC
CD
BD
BB
5
6
7
8
9
(b)
SHANK LENGTH (cm)

Table 46. Age at first egg (days)

Lines and crosses	No.	1968	1969	Av.	Dwarf effect[a]
BB	67	169	173	171	
BD	74	165	158	161	
*DB	68	169	166	167	6
*DD	31	165	182	174	
CC	111	165	170	168	
CD	40	164	165	164	
*DC	75	167	175	171	7

[a]Estimated number of days delay in maturity in the cross carrying the dwarf gene.

*Carry the dwarf gene.

Table 47. Percent hen-day egg production

Lines and crosses	1968	1969	Av.
BB	21.2	46.2	33.7
BD	55.6	63.1	59.4
*DB	51.5	64.0	57.8
*DD	42.4	51.7	47.1
CC	62.6	66.4	64.5
CD	52.7	76.0	64.3
*DC	62.1	60.2	61.2

*Carry the dwarf gene.

fertility and hatchability show that the dwarf gene had little effect on these traits also (Table 48).

Table 49 summarizes the effects of the dwarf gene. The effect was greater in the small line C diallel set than in the large line B diallel set for most traits. The dwarf gene effect on body weight was similar to that on shank length, but the effect on egg weight was less than on these other two traits.

Analysis of variance

The expectation of the sire line variance component, χ^2_s, is $(a + 2z)^2$ and that for the dam component, χ^2_d, is $(a + 2m)^2$; the interaction variance, χ^2_{Sd}, estimates the variance due to heterosis (h). The body weights at eight weeks of age in the B diallel set were adjusted for m so that the expected value of χ^2_d is a^2. These are considered as fixed effects and each has a single degree of freedom.

In the analyses of variance of body weights and shank length, the sire line mean squares were large mainly as a consequence of the dwarf gene effect (Tables 50 and 51). In particular, the line D sires transmitted the dwarf gene to their daughters in a hemizygous condition which reduced body size. The small dam line mean squares relative to the sire line mean squares are evidence that the maternal effect, m, was not important.

Analyses of variance of egg weights are given in

Table 48. Percent fertility and hatchability

Lines and crosses	Fertility			Hatchability[a]		
	1968	1969	Av.	1968	1969	Av.
BB	82.1	83.3	82.7	68.8	64.2	66.5
BD	76.5	92.5	84.5	69.2	83.3	76.2
*DB	75.6	89.8	82.7	70.2	73.2	71.7
*DD	61.6	92.3	76.9	35.2	64.9	50.1
CC	90.0	76.0	83.0	79.4	61.4	70.4
CD	86.4	87.6	87.0	76.1	76.6	76.3
*DC	83.6	85.7	84.7	75.9	76.8	76.3

[a]Percent of eggs set.

*Carry the dwarf gene.

Table 49. Summary of the dwarf effects[a] (%)

Trait	Age	Diallel set B	Diallel set C
Body wt.	10 days	-9.6	-12.4
	21 days	-12.4	-3.2
	8 wk	-35.6	-34.1
	22 wk	-29.7	-43.1
	32 wk	-24.5	-34.4
	50 wk	-27.1	-35.9
Shank length	22 wk	-27.4	-31.6
Egg wt.	32 wk	-7.5	-12.6
	50 wk	-4.4	-10.7
Age of first egg		+3.6	+4.1

[a]Change in the magnitude of each trait for the cross carrying the dwarf gene.

Table 50. Analyses of variance of body weights in females

Age (wk)	Source	d.f.	Mean square	
			Diallel set B	Diallel set C
8	Years	1	22561.1*	4087.9*
	Sire lines (S)	1	259250.4**	32811.5**
	Dam lines (D)	1	9300.7*	5862.3*
	S x D	1	4648.4	8114.8*
	Error	3	870.1	394.3
22	Years	1	0.00	0.01
	Sire lines	1	10.91**	1.15*
	Dam lines	1	1.88*	0.39
	S x D	1	0.11	0.15
	Error	3	0.06	0.07
32	Years	1	0.05	0.03
	Sire lines	1	15.09**	0.78*
	Dam lines	1	4.77**	0.44*
	S x D	1	0.01	0.23
	Error	3	0.05	0.03

**P < .01.
*P < .05.

Table 51. Analysis of variance of shank length in females at 22 weeks

Source	d.f.	Mean square	
		Diallel set B	Diallel set C
Years	1	0.42	0.75**
Sire lines (S)	1	16.53**	7.03**
Dam lines (D)	1	0.81	0.45*
S x D	1	0.34[a]	0.29*
Error	3	0.00	0.02

**P < .01.
*P < .05.

[a]Used as the error term with 4 d.f.

Table 52. The sire line mean squares were smaller relative to the dam line mean squares compared with those for body weights (Table 50). This indicates that the dwarf gene reduced egg weight relatively less than it reduced body weight. A similar conclusion is reached from Table 49. The significant sire by dam interaction indicates a heterosis effect on egg weight.

The error terms for sexual maturity and egg production were relatively large (Table 53). For egg production only two significant sources of variation were found. Heterosis seems to have an effect on both maturity and egg production in diallel set B.

Table 52. Analyses of variance of egg weight at two ages

Age (wk)	Source	d.f.	Mean square	
			Diallel set B	Diallel set C
32	Years (Y)	1	0.1*	3.5
	Sire lines (S)	1	263.3**	23.4*
	Dam lines (D)	1	109.6**	10.0
	S x D	1	15.5*	8.8
	S x Y	1	0.0	6.8
	Error	2	1.1[a]	1.3
50	Years	1	7.8	0.7
	Sire lines	1	373.4**	22.9**
	Dam lines	1	241.9**	2.7*
	S x D	1	11.0*	19.4**
	Error	3	1.1	0.1

**$P < .01$.
*$P < .05$.

[a]Three degrees of freedom.

Table 53. Analyses of variance of sexual maturity (age at first egg) and percent hen-day egg production

Trait	Source	d.f.	Mean square	
			Diallel set B	Diallel set C
Sex. mat.	Years	1	15.7	117.1
	Sire lines (S)	1	39.6	80.7
	Dam lines (D)	1	4.5	0.1
	S x D	1	124.8	17.4
	Error	3	56.3	25.4
Egg prod.	Years	1	371.1*	149.6
	Sire lines	1	69.0	211.3
	Dam lines	1	112.5	101.4
	S x D	1	659.5*	97.0
	Error	3	31.2	175.7

*$P < .05$.

Estimation of heterosis, autosome, and sex chromosome effects

Table 54 gives the parameter estimates for early body weights from the hatch 2, 1969, data. Heterosis ($\hat{h}$) increased early body weight, while the dwarf gene ($\hat{dw}$) decreased body weight in both diallel sets B and C. The autosome effect ($\hat{a}$) increased body weight in diallel set B, but decreased weight in diallel set C. This is as expected because the B line carried polygenes for large body size and the C line polygenes for small body size. The results for body weights, shank length, and egg weights taken at later ages shown in Tables 55 and 56 are similar to those in Table 54.

The estimated effects for the remaining traits are

Table 54. Estimates of heterosis ($\hat{h}$), autosome ($\hat{a}$), and dwarf ($\hat{dw}$) effects on early body weights (gm) of pullets from hatch 2, 1969

Age (days)	Diallel set B			Diallel set C		
	$\hat{h}$	$\hat{a}$	$\hat{dw}$	$\hat{h}$	$\hat{a}$	$\hat{dw}$
1	-0.4	0.6	0.0	0.1	-0.5	1.0
10	5.5	6.2	-4.2	5.6	-2.1	-4.4
21	11.0	10.1	-11.9	7.9	-3.1	-2.1

Table 55. Estimates of heterosis ($\hat{h}$), autosome ($\hat{a}$), and dwarf ($\hat{dw}$) effects on body weight, shank length, and egg weight at different ages in diallel set B

Trait	Age (wk)	$\hat{h}$	$\hat{a}$	$\hat{dw}$
Body wt. (gm)	8	24.1	68.2	-145.9
	22	53.3	442.7	-308.7
	32	11.4	701.0	-272.9
	50	49.9	858.0	-362.1
Shank length (cm)	22	0.12	0.64	-1.12
Egg wt. (gm)	32	1.4	7.4	-2.0
	50	1.2	11.0	-1.3

Table 56. Estimates of heterosis ($\hat{h}$), autosome ($\hat{a}$), and dwarf ($\hat{dw}$) effects on body weight, shank length, and egg weight at different ages in diallel set C

Trait	Age (wk)	$\hat{h}$	$\hat{a}$	$\hat{dw}$
Body wt. (gm)	8	31.9	-54.2	-91.1
	22	63.0	-201.1	-272.4
	32	76.7	-213.7	-248.6
	50	120.3	-168.0	-288.3
Shank length (cm)	22	0.17	-0.48	-1.18
Egg wt. (gm)	32	1.05	-1.20	-2.83
	50	1.13	-0.94	-2.16

presented in Table 57. The negative $\hat{h}$ values for sexual maturity imply that the crosses were heterotic for earlier maturity. In contrast, the dw allele evidently causes later sexual maturity. The a effect of -6.8 for egg production of set B seems to be mainly a reflection of the poor egg production (21.2 percent) of pure line B in 1968.

Feed efficiency

The means of traits involved in the feed efficiency test are given in Table 58. Efficiency of egg production was evaluated in three ways. In the first, it was estimated from the ratio $\bar{M}$ / $\bar{F}$ (Table 58). As expected, the BB hens had the lowest efficiency mainly because of their large body size. The dwarf gene in the high body weight line B back-

Table 57. Estimates of heterosis ($\hat{h}$), autosome ($\hat{a}$), and dwarf ($\hat{dw}$) effects on age of first egg, egg production, fertility, and hatchability

Trait	Diallel set B			Diallel set C		
	$\hat{h}$	$\hat{a}$	$\hat{dw}$	$\hat{h}$	$\hat{a}$	$\hat{dw}$
Sex. mat.	-3.9	1.50	3.0	-1.5	0.3	3.3
Egg prod.	9.2	-6.8	-0.8	3.5	7.2	-1.6
Fert.	1.1	3.6	-0.9	2.9	1.9	-1.2
Hatch.	7.8	6.0	-2.3	6.6	7.2	0.0

ground (*DB) increased efficiency of egg production presumably because of the reduction in body weight. The CC hens had the best feed conversion, even though they were not the smallest hens, but the differences in efficiency between the CC, CD, and *DC hens were not statistically significant.

The second estimate of efficiency was based on the multiple regression,

$$F = \hat{a} + \hat{b}_W W + \hat{b}_{\Delta W} \Delta W + \hat{b}_M M + e,$$

feed consumption (F) on initial body weight (W), change in body weight (ΔW), and egg mass produced per day (M). Efficiency was estimated as,

$$EF = \frac{\hat{b}_M \bar{M}}{\hat{b}_M \bar{M} + \hat{b}_W \bar{W}}.$$

The regression coefficients estimated across all periods

are given in Table 59. Change in body weight was taken into account in the analysis but was otherwise disregarded since ΔW was small and, hence, relatively unimportant. Thus, in effect, $\hat{b}_W$ and $\hat{b}_M$ were estimated free of any change in body weight, so that efficiency estimated from this multiple regression would not be influenced by ΔW. As expected, hens in the B group generally required less feed to maintain a unit of body weight than hens in the C group. The dwarf gene seemed to increase the feed required for egg production relative to that needed for body maintenance in the B group as shown by the +46.3 percent dwarf effect on the ratio $\hat{b}_M / \hat{b}_W$. The reverse was true for the C hens. The multiple regression estimates of feed requirements varied between feeding periods in both group B (Table 60) and group C (Table 61).

The EF values (Table 62) closely parallel the efficiencies given in Table 58. The third method, an indirect estimate of efficiency, $I = (\overline{M} / \overline{W})(k)$, seems to underestimate efficiency by one to five percent in the B group and overestimate it by about the same amount in the C group, but this may be a sampling effect. The indirect estimate accurately predicts the efficiency of one line or cross relative to that of another line or cross within each of the B and C groups. The utility of the indirect approach is that it does not require measurement of feed consumption.

Table 58. Data used for estimation of feed efficiency

Lines and crosses	No.	Body wt. (lb)	Change in body wt. (lb)	Hen-day egg prod. (%)	Egg mass per day (gm) $\bar{M}$	Feed consumed per day (gm) $\bar{F}$	Feed efficiency $\bar{M} / \bar{F}$
BB	73	6.6	0.26	55.2	33.1	126.1	0.26
BD	88	5.2	0.18	63.2	37.2	111.5	0.33
*DB	84	3.8	0.14	64.9	34.3	90.7	0.38
CC	81	2.4	0.04	69.3	29.6	67.9	0.44
CD	80	3.3	0.07	76.3	36.9	86.5	0.43
*DC	81	2.1	0.03	57.8	24.2	57.4	0.42

*Carry the dwarf gene.

Table 59. Estimates of feed requirements from multiple regression across all periods[a]

Lines and crosses	$\hat{b}_W$	$\hat{b}_M$	$\hat{b}_M/\hat{b}_W$	Dwarf effect (%)[b]
BB	9.2 ± 1.9	0.97 ± 0.10	0.106	
BD	12.2 ± 1.8	0.52 ± 0.11	0.043	
*DB	8.5 ± 1.5	0.68 ± 0.08	0.080	+46.3
CC	11.8 ± 4.9	0.66 ± 0.09	0.056	
CD	17.5 ± 1.6	0.74 ± 0.07	0.042	
*DC	15.9 ± 2.8	0.48 ± 0.09	0.030	-28.6
All	12.2 ± 0.2	0.81 ± 0.04	0.066	

[a]The estimates are: $\hat{b}_W$ = grams of feed required per pound of body weight per day; $\hat{b}_M$ = grams of feed required per day per gram of egg mass produced.

[b]Change in the ratio for the cross carrying the dwarf gene.

*Carry the dwarf gene.

Table 60. Estimates[a] of feed requirements from multiple regression for hens in the B diallel set, by periods

Lines and crosses	No.	Period	$\hat{b}_W$	$\hat{b}_M$
BB	28	1	15.3 ± 3.6	0.80 ± 0.19
	22	2	5.3 ± 3.2	1.15 ± 0.15
	23	3	10.5 ± 2.8	0.87 ± 0.22
BD	31	1	13.9 ± 2.5	0.68 ± 0.12
	28	2	13.2 ± 3.2	0.67 ± 0.15
	29	3	14.9 ± 3.3	1.00 ± 0.17
*DB	29	1	6.1 ± 3.0	0.65 ± 0.25
	28	2	6.0 ± 3.3	0.66 ± 0.13
	27	3	11.5 ± 2.1	0.96 ± 0.14

[a]The estimates are: $\hat{b}_W$ = grams of feed required per pound of body weight per day; $\hat{b}_M$ = grams of feed required per day per gram of egg mass produced.

*Carries the dwarf gene.

Table 61. Estimates[a] of feed requirements from multiple regression for hens in the C diallel set, by periods

Lines and crosses	No.	Period	$\hat{b}_W$	$\hat{b}_M$
CC	27	1	7.6 ± 6.3	0.88 ± 0.13
	28	2	18.9 ± 10.3	0.50 ± 0.14
	26	3	8.8 ± 6.4	0.59 ± 0.13
	27	1	15.4 ± 2.8	0.89 ± 0.12
	27	2	20.6 ± 2.8	0.59 ± 0.14
	26	3	13.8 ± 2.4	0.72 ± 0.12
*DC	27	1	13.1 ± 4.0	1.01 ± 0.12
	27	2	14.5 ± 4.2	0.37 ± 0.14
	27	3	7.6 ± 4.6	0.76 ± 0.15

[a]The estimates are: $\hat{b}_W$ = grams of feed required per pound of body weight per day; $\hat{b}_M$ = grams of feed required per day per gram of egg mass produced.

*Carries the dwarf gene.

Table 62. Feed efficiency estimated directly from multiple regression (EF) and indirectly from an index (I)

Lines and crosses	No.	$EF = \frac{\hat{b}_M \bar{M}}{\hat{b}_W \bar{W} + \hat{b}_M \bar{M}}$ [a]	$I = \frac{\bar{M}}{\bar{W}} (k)$ [b]
BB	73	0.24	0.19
BD	88	0.32	0.28
*DB	84	0.37	0.36
CC	81	0.45	0.49
CD	80	0.42	0.44
*DC	81	0.43	0.46

[a] Regression coefficients estimated across all lines and crosses ($\hat{b}_W = 12.2$, $b_M = 0.81$, Table 59).

[b] $k = \frac{(\overline{EF})(\bar{W})}{\bar{M}}$, where $\overline{EF}$, $\bar{W}$, and $\bar{M}$ were averaged across all lines and crosses.

*Carry the dwarf gene.

The partial correlations between the variables in the multiple regression analysis are listed in Table 63. The correlations between F and W and between F and M were usually high for all lines and crosses, as expected. As body weight increased, egg mass decreased slightly for the heavier hens ($r_{WM} = -0.14$, -0.12, and -0.09 for BB, BD, and *DB, respectively). The CC and *DC hen body weights seemed to be below the optimum for maximum egg mass, since the correlations between W and M (0.45 and 0.47) were relatively high ($p < .01$, 77 degrees of freedom). The high

Table 63. Partial correlations between variables in the feed efficiency test

Variables correlated[a]	Lines and crosses[b]					
	BB (69)	BD (84)	*DB (80)	CC (77)	CD (76)	*DC (77)
F (W)	0.32	0.57	0.45	0.53	0.54	0.59
F (ΔW)	0.42	0.41	0.43	0.29	0.47	0.25
F (M)	0.65	0.29	0.54	0.67	0.53	0.57
W (ΔW)	0.11	0.12	0.20	0.07	-0.02	-0.16
W (M)	-0.14	-0.12	-0.09	0.47	-0.03	0.45

[a]F = estimated feed consumed
W = initial body weight
ΔW = change in body weight
M = egg mass produced per day.

[b]Figures in parentheses are the numbers of degrees of freedom.

*Carry the dwarf gene.

correlations between rate of production (P) and feed conversion efficiency (FC), and between I and FC (Table 64), indicate that these variables are all reliable estimators of efficiency of feed utilization for egg production.

Table 64. Correlations between rate of production (P) and feed conversion efficiency FC = M/F and between P and an efficiency index I = M/W^a

Lines and crosses	Degrees of freedom	$r_{P(FC)}$	r_{PI}
BB	69	0.86	0.89
BD	84	0.84	0.80
*DB	80	0.88	0.86
CC	77	0.86	0.76
CD	76	0.78	0.83
*DC	77	0.84	0.80

[a]M = egg mass produced per individual hen per day,
F = feed consumed per hen per day,
W = initial body weight.

*Carry the dwarf gene.

DISCUSSION

A comparison of the results from different workers of the effect of the dwarf gene, dw, on several traits shows very good agreement (Table 65). In general, dwarfism reduces body weight and shank length each by about 30 percent while egg weight is reduced only about 10 percent. The effect of dwarfism on sexual maturity and rate of production seems to have been less consistent probably because of greater environmental influences. The evidence suggests that dwarf hens begin laying at a later age and produce at a lower rate. In broilers the dwarf effect on body weight, egg production, and egg weight closely parallels that in egg producing strains, but its effect on shank length seems to be less (Table 66).

Feed efficiency in terms of units of feed consumed per unit of egg mass produced for normal versus dwarf hens from different experiments is given in Table 67. Differences in efficiency can be accounted for by differences in body weight and rate of production or by sampling error.

Selvarajah et al. (1970) stated that "dwarfs have demonstrated remarkable feed efficiency in spite of their smaller body size and significantly lower feed intake as compared to the non-dwarfs". This conclusion seems to be highly questionable. First of all, the better feed efficiency of dwarfs is undoubtedly not "in spite of" but

Table 65. Estimates of dwarf gene effects in females of egg production strains at seventeen or more weeks of age

Relative dwarf effect[a] (%)				Days delay in sexual mat. due to *dw*	Author	Year
Body wt.	Shank length	Egg wt.	Egg prod.			
-29.0		-10.0	-15.3	24.7	Hutt	1959
-27.0		-10.0	-18.0		Bernier and Arscott	1960
-29.7	-29.4				Bernier and Arscott	1966
-30.0		-10.0			Merat	1969
-33.6	-25.0	- 8.5	-19.1		Mohammadian	1969
-31.4		- 9.6			Quisenberry *et al.*	1969
		-11.5	-13.8	11.0	Selvarajah *et al.*	1970
-36.8	-29.5	-10.1	- 3.8	6.5	ISU experiment	1968,1969

[a]Percent reduction in the trait of a cross carrying the dwarf gene compared to a reciprocal cross or a closely related non-dwarf.

Table 66. Estimates of dwarf gene effects in females of broiler strains

Proportion broiler inheritance	Relative dwarf effect[a] (%) Body wt. 8 wk.	Body wt. 22-26 wk.	Shank length 8 wk.	Shank length 22-26 wk.	Egg prod.	Egg wt.	Author	Year
3/4	-36.5	-29.3		-21.0	0.0	-9.5	Mohammadian	1969
7/8	-23.5	-21.3		-18.4	-13.4	-7.6		
15/16	-24.4			-20.0				
3/4	-27.0	-23.8	-12.0	-14.9			ISU data	1968, 1969
7/8	-29.4	-30.8	-15.2	-19.2				

[a]Percent reduction in the trait of a cross carrying the dwarf gene compared to a closely related non-dwarf.

Table 67. Feed efficiency of dwarfs versus non-dwarfs

Feed efficiency[a]			
Dwarfs	Non-dwarfs	Author	Year
2.7	2.4	Magruder and Coune	1969
3.2	3.9	Prod'homme and Merat	1969
2.5	2.6	Quisenberry et al.	1969
2.5	2.8	Selvarajah et al.	1970
2.5	2.7	ISU experiment	1968,1969

[a]Units of feed consumed divided by units of egg mass produced.

"because of " lower body weight and, consequently, lower requirement for body maintenance. Dwarfs and non-dwarfs of the same body weight probably have equal feed efficiency as deduced from our study. This is supported by Guillaume (1969) who found that dwarf pullets during the growing period consumed as much feed as normal pullets of the same body weight. Similarly, Bernier and Arscott (1966) found that dwarfs averaging 72 percent of the body weight of normal pullets consumed 70 percent as much feed. Efficiency of gain in weight and of protein utilization may be lower in dwarf than in normal pullets, although dwarfs may be more efficient in energy utilization (Guillaume, 1969). Also, the percentage difference in body weight between adult dwarf

and non-dwarfs seems to be nearly proportional to the difference in feed consumption (Arscott and Bernier, 1968; Prod'homme and Merat, 1969). In the present study, dwarf DC pullets weighed 34 percent less than the reciprocal cross CD hens and consumed 34 percent less feed, while the dwarf DB hens weighed 25 percent less than the non-dwarf BD hens and consumed 19 percent less feed. Furthermore, the pure line CC hens and the dwarf DC hens were nearly equal in feed efficiency. The slight difference in efficiency between these two groups was probably caused by sampling variation. Hence, polygenes favoring small body size in line C seemed to have the same effect on efficiency as the major gene for small body size, dw. Prod'homme and Merat (1969) found nearly equal feed efficiency for dwarf and normal hens when comparisons were made in terms of multiple regression of feed consumption on body weight, change in weight, and egg weight. In summary, when all of the available information is considered, dwarf chickens are probably not unique in their high feed efficiency: any advantage they have seems to be wholly a consequence of their reduced body size.

The validity of the statistical model tested in this study required several underlying assumptions. First, maternal effects, for all traits except body weight to eight weeks of age in diallel set B, were assumed to be absent. There could be a small maternal influence on body

weights at later ages and perhaps also on egg weight, age at first egg, and percent hen-day production (Hazel and Lamoreux, 1947; Düzgünes and Yao, 1956; King, 1961; Goodwin et al, 1964). It was also assumed that the W sex chromosome was genetically inert. However, Briggs (1970) found evidence that the W chromosome may exert some influence on eight-week body weight, 22-week shank length, and 32-week egg weight. Finally, the assumption was made that differences in effects of the Z sex chromosome were wholly accounted for by the dwarf gene locus. Briggs (1970) showed that different Z chromosomes with the normal allele for non-dwarf influenced age at first egg, and possibly also egg production and eight-week body weight.

The economic value of egg producing strains of chickens is a function of rate of production, age at maturity, egg weight, and body weight, as well as cost of replacement pullets and density of hens in the laying house. Egg production and sexual maturity of dwarf hens seem to be somewhat inferior to those of normal hens. Supplementary selection among the dwarfs to improve these traits might help to solve this problem, yet a delay in age of first egg seems to persist for at least one commercial breeding firm.[1] The

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[1]Munro, S. S. Hy-Line Poultry Farms, Johnston, Iowa. Private communication. 1970.

maintenance of satisfactory market egg weight is also important. For example, based on 50-week egg weight in this study, the average weights in ounces per dozen eggs were:

Large Leghorn group	BB	29.0
	BD	25.3
	*DB	24.1
Small Leghorn group	CC	19.4
	CD	21.4
	*DC	19.1

where the asterisk denotes the dwarf hens. Both the dwarf and non-dwarf hens of the large Leghorn line group laid eggs large enough to grade large. Hence, the price per dozen would be nearly the same for both groups, except that a slightly larger proportion of the eggs from the non-dwarfs than from DB would be extra large instead of large. Thus, it would be expected that the dwarf hens would be more profitable because of their smaller size and, hence, greater feed efficiency (Table 58). The hens of all three groups in the C diallel set laid small to medium sized eggs. Therefore, the CC and DC hens, being the smallest, might be expected to be the most profitable within the C group.

The net economic return from a particular strain of laying hens would also be a function of the cost of replacement pullets and of the stress factor caused by the density level of the hens in the flock. Smaller pullets have less rearing costs and are able to tolerate a higher laying flock density. The higher the bird density the greater the efficiency of use of equipment, building space, and labor.

Line C hens and DC dwarf hens probably would differ little in rearing cost or in tolerance against stress from limiting space. Perhaps the shorter shanks of the dwarfs might allow for somewhat smaller and lower cost cages. Although egg production per bird would be lower with more than one bird per cage (Rose and Sell, 1969; Wildey et al, 1969), income per unit of cage and feed efficiency seem to be greatest with more than one bird per cage (Champion and Zindel, 1968; Quisenberry et al, 1969; Ruszler and Quisenberry, 1969).

Using the data in Table 58, the number of eggs of an arbitrary size of 60 grams expected from hens of each of the six genetic groups was estimated (Table 68). The number of hens which would utilize the same feed equivalent consumed by 100 BB hens per day (12.61 kilograms) would be, for example, 113 for the cross BD. The results indicate that the non-dwarf small body size CC hens would require the least amount of space to produce the most eggs (92).

Broiler breeder hens are expensive to maintain because they have high feed costs. The possible advantages of dwarf broiler hens over normal sized hens include higher feed efficiency, fewer double yolked eggs, better shell quality, and higher hatchability (Jaap, 1969; Jaap and Mohammadian, 1969; "Midget" broiler making progess, 1971). Also, dwarf hens would require less floor space because of their smaller size. The most serious disadvantage would be the expected

Table 68. Hypothetical number of hens and number of 60-gram egg equivalents expected from the different genetic groups each fed the same amount of feed per day

Lines and crosses	No. of hens	Feed required per day (kg)	Feed efficiency[a]	Expected total egg mass output per day (kg)	Expected no. of eggs of equivalent 60-gram size produced per day
BB	100	12.61	0.26	3.31	55
BD	113	12.61	0.33	4.31	69
DB	139	12.61	0.38	4.67	78
CC	186	12.61	0.44	5.54	92
CD	146	12.61	0.43	5.42	90
DC	220	12.61	0.42	5.29	88

[a]From Table 58, egg mass produced per day divided by feed consumed per day.

reduction in broiler growth rate. Mohammadian (1970) found about three percent lower eight-week body weight in broilers produced from mating normal broiler sires to dwarf dams.

SUMMARY

The nature and effects of two types of major genes in chickens were investigated in this study. The first consisted of genes determining serum protein allotypes and the second was a sex-linked gene causing dwarfism. The latter was studied mainly as it related to economically important traits of the chicken.

Allotypes are genetically determined differences in serum protein isoantigens. In part I of the study, three subpopulations of allotype chickens, A-a, A-b, and A-c, were combined in an attempt to produce a single population segregating only for known allotype alleles. Individuals of selected allotypes were mated over two generations, but further generations will yet be needed to eliminate unknown allotype genes. About 600 progeny were included in the study. Subpopulation A-a segregated for the alleles a^1 and a^2 and A-b for b^1 and b^2. Based on an earlier study, the population A-c was assumed to segregate for four allotypic specificities, c1, c2, c3, and c4, but the present study indicated that either the c allotypes were not determined by alleles segregating at a single locus or they are determined by a more complex phenogroup type of inheritance.

A problem of a technical nature involved the question of reproducing the set of anti-allotype sera used as standards for allotyping unknown sera. In order to make it

possible to allotype succeeding generations, either large amounts of each reagent would be initially required to be on hand or identical allotypic reagents must be reproducible as the initial supply becomes exhausted. Efforts were made to produce new antisera first by isoimmunization. Fifty-five donor anti-Brucella abortus antisera were mixed with the homologous antigen and injected subcutaneously with Freund's incomplete adjuvant into 233 recipients, producing 39 antisera. About two-thirds of these seemed to contain antibodies against allotypes; at least one was a reproduction of an identified antiserum (c1). Antibodies against other known allotypes may have been produced, but they could not be positively identified. A few of the precipitins may have been directed against idiotypes (antigenic determinants unique to a particular antibody, in this case anti-Brucella).

Another antiserum was obtained by injecting six recipients with a complex similar to that indicated above, but by substituting anti-Brucella egg yolk globulin for anti-Brucella serum. The identity of two antisera made from acid-precipitated donor serum proteins was not determined, but they seemed not to contain specificities for known allotypes. Two antisera developed from heteroimmunizations in two turkey recipients, following essentially the same procedure as for the isoimmunizations, seemed to contain mostly antibodies against species-specific antigens.

Differences between donor sera in immunogenicity of allotypic antigens and the effect of donor and recipient origin, and of frequency of injection, on antiserum production was also investigated. Donors apparently differed in antigenicity of different allotype antigens; chickens from the A-b subpopulation seemed to be more effective donors than those from A-a and A-c. Close family relationship seemed to have a negative effect on antiserum production. Injection frequency was evidently important in the success of making an antiserum.

Whether the allotype genes can be fully utilized in poultry breeding practice will depend first on whether uniform typing reagents can be reproduced and maintained.

Part II of the study was a genetic analysis of the effect of the sex-linked recessive dwarfism gene, dw, on economically important quantitative traits. A large Leghorn line B and a small Leghorn line C were each reciprocally crossed with a dwarf line D carrying the dwarf gene dw. Lines B and C carried polygenes for large and small body size, respectively, while line D was assumed to carry polygenes for intermediate body size. Approximately 100 to 150 pullet records per line or cross were taken on body weight at eight and 22 weeks and on shank length at 22 weeks over two years. Records on about 80 hens per line or cross were kept to measure age at sexual maturity, rate of egg produc-

tion, body wieght, and egg weight. Means were calculated across all individuals and years and summarized in 2x2 tables for each trait within each of the B and C cross groups. From these, three effects were estimated: an autosomal effect associated with each of the lines B, C, and D carrying polygenes for large, small, and intermediate body size, respectively; a sex chromosome effect assumed to be determined entirely by the dwarf gene dw; and the effect of heterosis caused by the interaction of genes of the sire line mated to the dam line.

The autosomal effects of the B line increased body weight, egg weight, and shank length, while those of the C line favored a decrease. Heterosis effects produced higher body weight, egg weight, shank length, and rate of production, and earlier maturity, in both the B and the C crosses. The dwarf gene dw reduced body weight and shank length about 30 percent and egg weight about 10 percent, while its effect on egg production, fertility, and hatchability was small and inconsistent. Age at first egg in the dwarf pullets was about one week later than in the reciprocal cross normals. A maternal effect due to egg size of the dam parent was found for juvenile body weights in the B crosses.

Feed efficiency of dwarfs compared to normals was also studied. Efficiency was estimated in three ways. The first was $FC = \bar{M} / \bar{F}$, where $\bar{M}$ and $\bar{F}$ are the egg mass and feed

consumption means for each line or cross. In the second, a multiple regression equation was used. Daily feed consumption (F) was regressed on initial body weight (W), change in body weight (ΔW), and egg mass produced per day (M). Efficiency was then estimated as $EF = (\hat{b}_M\bar{M}) / (\hat{b}_M\bar{M} + \hat{b}_W\bar{W})$, where $\hat{b}_M$ is the regression of feed required for egg production, $\hat{b}_W$ the regression of feed required for body maintenance, and $\bar{M}$ and $\bar{W}$ are the egg mass and body weight means for each line or cross. The third estimate of efficiency was an indirect measure, $I = \bar{M} / \bar{W}$ and did not require prior information on feed consumption. The indirect estimate proved to be nearly as accurate as EF and FC in measuring the efficiency of one line or cross relative to that of another within each of the B and C groups. Feed efficiency was nearly the same for both the pure line C and the cross line dwarf DC hens, indicating that polygenes for low body weight have essentially the same relative effect on efficiency as the dwarf gene.

The value of the dwarf gene in improving egg production strains depends largely on whether it has certain unique properties not associated with body weight reduction in enhancing the efficiency of egg production. The results of this study indicate that use of the dwarf gene and conventional selection for low body size both lead to essentially the same results.

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APPENDIX

Table 69. Matings of allotype subpopulations (1969, P_0)

Sire no.	Origin	Genotype[a,b]	Dam no.	Origin	Genotype[a,b]
2437	A-a	$a^2a^2\ b^1b^2\ c^1c^1$	18748	A-a	$a^1a^2\ b^1b^2\ c^1c^4$
			18884	A-b	$a^2/-\ b^1b^2\ c^1c^2$
2412	A-a	$a^1a^1\ b^1b^1\ c^2c^4$	2449	A-a	$a^1a^2\ b^1b^2\ c^1/-$
			18729	A-c	$a^1a^2\ b^1b^2\ c^2c^3$
			18888	A-b	$a^1a^2\ b^1b^2\ (c^1)c^2c^3$
2417	A-a	$a^1a^1\ b^1b^2\ c^2c^4$	2462	A-a	$a^1a^2\ b^1/-\ c^1c^4$
			2487	A-a	$a^1a^2\ b^1b^2\ c^1c^4$
			2497	A-a	$a^1/-\ -/-\ c^1c^4$
			18864	A-b	$a^1/-\ b^1b^2\ c^3c^4$
2419	A-a	$a^1a^2\ b^1b^2\ c^1c^4$	18875	A-b	$-/-\ b^1b^2\ c^2c^4$
			18905	A-b	$-/-\ b^2b^2\ c^1c^2$
2445	A-a	$a^2/-\ b^1b^2\ c^1c^4$	2402	A-a	$a^1a^2\ b^1/-\ c^1c^4$
			2475	A-a	$a^1/-\ -/-\ c^4/-$
			2494	A-a	$a^2/-\ b^1b^2\ c^1c^2$
			18865	A-b	$a^1/-\ b^1/-\ c^1c^3$
			18879	A-b	$a^2/-\ b^1b^1\ (c^1)c^2c^3$

[a]The dash (e.g., $a^1/-$) means the allele is unidentified, or the individual could be homozygous for the known allele.

[b]Genetics of the c allotypic system were questionable; c1 seemed to be the "extra" antigen.

Table 69 (Continued)

Sire no.	Origin	Genotype[a,b]	Dam no.	Origin	Genotype[a,b]
2451	A-a	$a^1/-\ b^1b^2\ c^1/-$	2486	A-a	$a^1/-\ b^1b^2\ c^1c^4$
			18728	A-c	$a^1a^2\ b^1b^1\ c^1c^3$
			18870	A-b	$a^2/-\ b^1/-\ c^1c^4$
			19085	A-b	$a^2/-\ b^1b^2\ c^1c^4$
2500	A-a	$a^2/-\ b^1b^2\ c^1c^2$	2468	A-a	$a^2/-\ b^1b^2\ (c^1)c^2c^4$
			19100	A-b	$a^1/-\ b^2/-\ c^1c^3$
18739	A-c	$a^1a^2\ b^1b^2\ c^1c^4$	2409	A-a	$a^1a^2\ -/-\ c^1c^2$
			2455	A-a	$a^1a^2\ b^1b^2\ c^1c^4$
			2517	A-a	$a^2/-\ b^1b^2\ c^1c^1$
			18902	A-b	$a^1a^2\ b^2b^2\ c^1c^4$
18876	A-b	$a^2/-\ b^1b^2\ c^3c^4$	2425	A-a	$a^2/-\ b^1b^2\ c^1c^1$
			2482	A-a	$a^2/-\ b^1b^2\ c^1c^1$
18885	A-b	$a^2/-\ b^1b^2\ c^1c^1$	2450	A-a	$a^1/-\ -/-\ c^4/-$
			2477	A-a	$a^1/-\ -/-\ c^1c^4$
			2483	A-a	$a^2/-\ -/-\ c^1/-$
			2506	A-a	$a^2/-\ b^1b^2\ c^1/-$
18898	A-b	$-/-\ b^2b^2\ c^1c^4$	2413	A-a	$a^1a^1\ -/-\ c^2/-$
			2466	A-a	$a^2/-\ -/-\ c^1/-$
			2527	A-a	$a^1a^2\ b^1b^2\ c^1c^2$

Table 69 (Continued)

Sire no.	Origin	Genotype[a,b]	Dam no.	Origin	Genotype[a,b]
19102	A-b	$a^1/-\ b^1b^2\ (c^1)c^3c^4$	2416	A-a	$a^1/-\ b^2/-\ c^2c^4$
(18756)[c]	A-c	$a^1/-\ b^1/-\ c^2c^4$	2498	A-a	$a^2/-\ b^1/-\ c^1c^4$
19112	A-b	$a^1/-\ b^1b^2\ (c^1)c^2c^3$	2484	A-a	$a^2/-\ b^1b^2\ c^1c^1$
			2515	A-a	$a^1a^1\ -/-\ c^1c^4$
			2520	A-a	$a^2/-\ b^1/-\ c^1/-$
18903	A-b	$a^1/-\ b^2/-\ (c^1)c^3c^4$	2511	A-a	$a^2a^2\ b^1b^2\ c^1c^1$
			18718	A-c	$a^1a^1\ b^2b^2\ c^2c^4$
			18890	A-b	$a^1a^2\ b^1/-\ c^1c^4$
			19113	A-b	$a^1/-\ b^1/-\ c^1/-$
19102	A-b	$a^1/-\ b^1b^2\ (c^1)c^3c^4$	2516	A-a	$a^1/-\ b^1b^1\ c^1c^4$
(18906)[c]	A-b	$a^2/-\ b^2/-\ c^4c^4$			

[c]Male 19102 died after one insemination and was replaced by 18756 and 18906.

Table 70. Allotype matings (1970, P_1)

Sire no.	Genotype[a,b]	Dam no.	Genotype[a,b]
20253	$a^1a^2\ b^1b^2\ c^1c^4$	20368	$a^2/-\ b^1/-\ c^1c^3$
		20378	$a^2a^2\ b^1b^1\ c^1c^3$
		20440	$a^2/-\ b^1b^1\ c^1c^1$
		20491	$a^1a^1\ b^1b^1\ c^4c^4$
		20497	$a^1/-\ b^1b^2\ -/-$
20257	$a^1a^1\ b^1/-\ c^4c^4$	20239	$a^1/-\ b^1b^1\ (c^1)c^3c^4$
		20411	$a^1a^2\ b^2/-\ c^4/-$
		20473	$a^1a^2\ b^2/-\ c^1c^3$
		20490	$a^1a^2\ b^1b^2\ c^1c^4$
20263	$a^1a^1\ b^1b^2\ c^4c^4$	20242	$a^1/-\ b^1/-\ (c^1)c^3c^4$
		20369	$a^2/-\ b^1b^1\ c^1c^3$
		20419	$a^1a^2\ b^2/-\ \ c^3c^4$
		20486	$-/-\ b^1/-\ c^1c^3$
20374	$a^2a^2\ b^1/-\ (c^1)c^3c^4$	20311	$a^1a^2\ b^1b^2\ c^3c^4$
		20322	$a^1a^2\ b^1/-\ -/-$
		20404	$a^2/-\ b^1b^2\ c^1c^1$
		20442	$a^1a^2\ b^1b^2\ c^1c^3$

[a]The dash (e.g., $a^1/-$) means the allele is unidentified, or the individual could be homozygous for the known allele.

[b]The genetics of the c allotypic system were questionable; c1 seemed to be the "extra" antigen.

Table 70 (Continued)

Sire no.	Genotype[a,b]	Dam no.	Genotype[a,b]
20376	$a^2/-\ b^1b^1\ c^1c^3$	20221	$a^1a^2\ b^1/-\ c^1\ c^4$
		20274	$a^1a^1\ b^1b^2\ (c^1)c^3c^4$
		20456	$a^1/-\ b^1/-\ (c^1)c^3c^4$
		20457	$a^1/-\ b^1b^2\ (c^1)c^3c^4$
20476	$a^1a^2\ b^2/-\ c^4/-$	20201	$a^1a^2\ b^1b^2\ c^1c^4$
		20234	$a^1/-\ b^1/-\ (c^1)c^3c^4$
		20238	$a^1a^2\ b^1/-\ c^1c^4$
		20241	$a^1a^2\ b^1/-\ c^1c^4$
20485	$a^1a^2\ b^2/-\ (c^1)c^3c^4$	20367	$a^2a^2\ b^1b^1\ c^1c^3$
		20430	$a^1/-\ b^2/-\ c^4/-$
20492	$a^1a^1\ b^1b^1\ c^4c^4$	20251	$a^1a^2\ b^1b^2\ c^1c^4$
		20256	$a^1a^2\ -/-\ c^1/-$
		20270	$a^1a^2\ b^1/-\ c^4/-$
		20275	$a^1a^2\ b^1b^2\ c^4/-$
20495	$a^1/-\ b^1b^2\ (c^1)c^3c^4$	20264	$a^1a^2\ -/-\ c^1/-$
		20273	$a^1/-\ b^1/-\ c^3/-$

Table 70 (Continued)

Sire no.	Genotype[a,b]	Dam no.	Genotype[a,b]
20499	$a^1a^2\ b^1b^2\ c^4c^4$	20240	$a^1a^1\ b^1b^1\ (c^1)c^3c^4$
		20243	$a^1a^1\ b^1b^1\ (c^1)c^3c^4$
		20254	$a^1a^2\ b^1b^2\ c^1\ c^4$
		20255	$a^1a^2\ b^1/-\ c^1/-$
		20269	$a^1a^1\ b^1/-\ c^3c^4$
		20272	$a^1a^1\ b^1b^1\ c^3c^4$

Table 71. Injections in 1968

Donor		Recipient	
Wingband no.	Phenotype[a]	Wingband no.	Phenotype[a]
5822	b1	5811	b2
		5823	b2
		5833	b2
		5834	b2
5823	b2	5801	b1
		5822	b1
		5825	b1
5833	b2	5799	b1
		5806	b1
		5810	b1
		8367	a2
5939	c2	5948	c1
		5952	c1
		6003	c1
		6006	--
		6132	--
		6148	c1
5950	c4	5969	c3
		6128	--
		20076	a2

[a]Typed for only one locus; dashes mean no identified antigens present. The letter designating the phenotypic system corresponds to the subpopulation origin; for example, donor 5822 was from subpopulation A-b, while 5939 was from A-c.

Table 71 (continued)

Donor		Recipient	
Wingband no.	Phenotype	Wingband no.	Phenotype
5952	c1	5939	c2
		5945	--
		5950	c4
6003	c1	5951	--
		5998	--
		6141	c2
20076	a2	8535	a1
		20004	a1
		20051	--
		20052	--
		20053	--
		20060	a1
		20061	--
		20071	a1
W196	c1	S-lines	unknown
LW281	c2	S-lines	unknown

Table 72. Injections in series I, 1969

Donor			Recipient			Antisera sought
Wingband no.	Phenotype	Origin	Wingband no.	Phenotype	Origin	
2442	a2 c1	A-a	2418	a1 c1 c4	A-a	a2
			2433	c1	A-a	a2
			2465	c1	A-a	a2
			2509	c1	A-a	a2
			18887	c1 c2	A-b	a2
			19098	c1 c2	A-b	a2
2427	a2 c1	A-a	2517	b1 c1	A-a	a2
			18718	b2 c2 c4	A-c	a2 c1
2509	c1	A-a	2475	a1 c4	A-a	c1
			2504	b1	A-a	c1
			18716	a1 c2	A-c	c1
18716	a1 c2	A-c	2497	a1 c1	A-a	c2
			2515	a1 c1 c4	A-a	c2
18733	b1 b2 c2 c4	A-c	2426	b1 b2 c1	A-a	c2
			2468	a2 b1 b2 c1 c4	A-a	
			18906	a2 b2 c4	A-b	b1 c2
18871	b1	A-b	2440	c1	A-a	b1
			19091	c1	A-b	b1

Table 72 (Continued)

Donor			Recipient			Antisera sought
Wingband no.	Phenotype	Origin	Wingband no.	Phenotype	Origin	
18874	b1 b2 c2 c4	A-b	2511	a2 b1 b2 c1	A-a	c2 c4
			18879	a2 b1 c1 c2 c3	A-b	b2 c4
			18898	b2 c1 c4	A-b	b1 c2
			19094	b1 b2 c2	A-b	c4
18887	c1 c2	A-b	2476	a2 c1	A-a	c2
			2483	a2 c1	A-a	c2
			2519	a2 c1	A-a	c2
18896	a1 b2 c1 c3	A-b	2420	a1 b1 c1 c4	A-a	b2 c3
			2460	a1 b1 c1 c3	A-a	b2
			2463	a1 b1 c1 c4	A-a	b2 c3
			2489	a2 b1 c1 c2	A-a	a1 b2 c3

Table 73. Injections in series II, 1969

Donor			Recipient			Antisera sought[a]
Wingband no.	Phenotype	Origin	Wingband no.	Phenotype	Origin	
2425	a2 b1 b2 c1	A-a	2510	a2 b1 c1	A-a	b2
			18902	a1 a2 b2 c1 c4	A-b	b1
2434	a2 b1 b2 c1	A-b	2507	a1 b1 b2 c1 c4	A-a	a2
			2508	b1 b2 c1	A-a	a2
			18878	b1 b2	A-b	a2 c1
			2205	unknown	Dwx	
			5208	unknown	Dwx	
2437	a2 b1 b2 c1	A-a	8417	a2		b1 b2 c1
			8427	a2		b1 b2 c1
			19112	a1 b1 b2 c1 c2	A-b	a2
			19113	a1 b1 c1	A-b	a2 b2
2466	a2 c1	A-a	18897	a1 c1 c4	A-b	a2
			19078	a1 c1 c3	A-b	a2
5834	b2	A-b	2259	unknown	Dwx	
			5152	unknown	Dwx	
			5209	unknown	Dwx	
			5240	unknown	Dwx	

[a]The phenotypes of Dwx recipients were unknown; hence, the antisera sought was indefinite.

Table 73 (Continued)

Donor			Recipient			Antisera sought
Wingband no.	Phenotype	Origin	Wingband no.	Phenotype	Origin	
18879	a2 b1 c1 c2 c3	A-b	2262	unknown	Dwx	
			5063	unknown	Dwx	
			18733	b1 b2 c2 c4		a2 c1 c3
			18874	b1 b2 c2 c4		a2 c1 c3
18906	a2 b2 c4	A-b	2147	unknown	Dwx	
			2466	a2 c1	A-a	b2 c4
			2498	a2 b1 c1 c4	A-a	b2
19080	a2 b1 c1 c2	A-b	2485	a2 b1 b2 c1	A-a	c2
			18756	a1 b1 c2 c4	A-c	a2 c1

Table 74. Injections in series III, 1969

Donor		Number of recipients[b]
Wingband no.	Phenotype[a]	
20060	a1	4
8367	a2	4
5801	b1	4
5822	b1	5
5811	b2	6
5952	c1	1
6003	c1	4
5939	c2	5

[a]The letter designating the phenotypic system corresponds to the subpopulation origin; for example, 20060 was from subpopulation A-a.

[b]Phenotypes of recipients (GW, S-lines) were unknown.

Table 75. Injections in series IV, 1969

Donor		Number of recipients[b]
Wingband no.	Phenotype[a]	
20076	a2	5
5810	b1	4
5823	b2	6
5833	b2	6
5948	c1	2
6148	c1	5
6141	c2	5
5950	c4	6

[a]The letter designating the phenotypic system corresponds to the subpopulation origin; for example, 20076 was from subpopulation A-a.

[b]Phenotypes of recipients (GW,WG,W,S-line) were unknown.

Table 76. Injections in series V, 1969

Donor			Recipient			Antisera sought[a]
Wingband no.	Phenotype	Origin	Wingband no.	Phenotype	Origin	
2412	a1 b1 c2 c4	A-a	93	unknown	I-9	
			102	unknown	I-9	
			298	unknown	I-GH	
			675	unknown	I-19	
			696	unknown	I-19	
2417	a1 b1 b2 c2 c4	A-a	59	unknown	I-9	
			212	unknown	I-19	
			2402	a1 a2 b1 c1 c4	A-a	b2 c2
			2416	a1 b2 c2 c4	A-a	b1
2419	a1 a2 b1 b2 c1 c4	A-a	71	unknown		
			228	unknown		
			293	unknown		
			2409	a1 a2 c1 c2	A-a	b1 b2 c4
			2413	a1 c1 c2	A-a	a2 b1 b2 c4
2439	a2 b1 b2 c1	A-a	94	unknown	I-9	
			668	unknown	I-19	
			2427	a2 c1	A-a	b1 b2
			2466	a2 c1	A-a	b1 b2
2445	a2 b1 b2 c1 c2 c4	A-a	86	unknown	I-9	
			265	unknown	I-GH	
			2439	a2 b1 b2 c1	A-a	c2 c4

[a]Phenotypes of the inbreds were unknown; hence, the expected antisera were indefinite.

Table 76 (Continued)

Donor			Recipient			Antisera sought[a]
Wingband no.	Phenotype	Origin	Wingband no.	Phenotype	Origin	
2457	a2 b1 b2 c1 c2	A-a	2434	a2 b1 b2 c1	A-a	c2
			2437	a2 b1 b2 c1	A-a	c2
			2449	a1 a2 b1 b2 c1	A-a	c2
			2453	a1 a2 b1 c1 c2	A-a	b2
			2462	a1 a2 b1 c1	A-a	b2 c2
			2468	b1 b2 c1 c2	A-a	a2
2498	a2 b1 c1 c4	A-a	2482	a2 b1 b2 c1	A-a	c4
			2484	a2 b1 b2 c1	A-a	c4
			2486	a1 b1 b2 c1	A-a	a2 c4
			2502	a1 c1	A-a	a2 b1 c4
			2516	a1 b1 c1 c2	A-a	a2 c4
			2520	a2 b1 c1	A-a	c4
18875	b1 b2 c2 c4	A-b	74	unknown	I-9	
			225	unknown	I-19	
			290	unknown	I-GH	
			670	unknown	I-19	
18879	a2 b1 c1 c2 c3	A-b	272	unknown	I-GH	
			690	unknown	I-19	
			18864	a1 b1 b2 c3 c4	A-b	a2 c1 c2
			18870	a2 b1 c1 c4	A-b	c2 c3
			18880	a2 b1 b2 c1 c4	A-b	c2 c3
			18884	a2 b1 b2 c1 c2	A-b	c3
			18887	c1 c2	A-b	a2 b1 c3

Table 76 (Continued)

Donor			Recipient			Antisera sought[a]
Wingband no.	Phenotype	Origin	Wingband no.	Phenotype	Origin	
18898	b2 c1 c4	A-b	4	unknown	I-GH	
			83	unknown	I-9	
			203	unknown	I-19	
			352	unknown	I-GH	
			697	unknown	I-19	
			18865	a1 b1 c1 c3	A-b	b2 c4
			18897	a1 c1 c4	A-b	b2
			18900	a2 c1 c4	A-b	b2
18902	a1 a2 b2 c1 c4	A-b	689	unknown	I-19	
			18888	a1 a2 b1 b2 c1 c2 c3	A-b	c4
			19083	a1 b1 b2 c3 c4	A-b	a2 c1
			19106	a2 b1 c1 c4	A-b	a1 b2
18906	a2 b2 c4	A-b	276	unknown	I-GH	
			645	unknown	I-19	
			651	unknown	I-19	
			19078	a1 c1 c3	A-b	a2 b2 c4
			19079	b1	A-b	a2 b2 c4

Table 77. Allotyping test results for the F_1 - 1969

Parents - P_0				Offspring - F_1	
Sire no.	Deduced genotype[a,b]	Dam no.	Deduced genotype[a,b]	Wingband no.	Phenotypic test outcome[b]
2437	$a^2a^2\ b^1b^2\ c^1c^1$	18748	$a^1a^2\ b^1b^2\ c^1c^4$	20201	a1 a2 b1 b2 c1 c4
				20202	a1 a2 b1 b2 c1 c4
				20205	a1 a2 b1 c1 c4
				20208	a1 a2 b1 c1
				20209	a1 a2 b1 c1
		18884	$a^2/-\ b^1b^2\ c^1c^2$	20214	a2 b1 b2 c1
				20216	a2 b1 b2 c1 c4
2412	$a^1a^1\ b^1b^1\ c^2c^4$	2449	$a^1a^2\ b^1b^2\ c^1/-$	20221	a1 a2 b1 c1 c4
				20226	a1 a2 b1 c1
				20227	a1 b1 c1 c4
		18888	$a^1a^2\ b^1b^2\ (c^1)c^2c^3$	20234	a1 b1 (c1) c3 c4
				20238	a1 a2 b1 c1 c4
				20239	a1 b1 (c1) c3 c4
				20240	a1 b1 (c1) c3 c4
				20241	a1 a2 b1 c1 c4
				20242	a1 b1 (c1) c3 c4
				20243	a1 b1 b2 (c1) c3 c4
				20244	a1 a2 b1 c1 c3

[a]The dash (e.g., $a^1/-$) means the allele is unidentified, or the individual could be homozygous for the known allele.

[b]Genetics of the c allotypic system were questionable; c1 seemed to be the "extra" antigen.

Table 77 (Continued)

Parents - P_0				Offspring - F_1						
Sire no.	Deduced genotype[a,b]	Dam no.	Deduced genotype[a,b]	Wingband no.	Phenotypic test outcome[b]					
2417	$a^1a^1\ b^1b^2\ c^2c^4$	2462	$a^1a^2\ b^1/-\ c^1c^4$	20250	a1	a2	b1		c1	
				20251	a1	a2	b1	b2	c1	c4
		2487	$a^1a^2\ b^1b^2\ c^1c^4$	20253	a1	a2	b1	b2	c1	c4
				20254	a1	a2	b1	b2	c1	c4
				20255	a1	a2	b1		c1	c4
				20256	a1	a2			c1	c4
				20257	a1		b1			c4
		2497	$a^1/-\ -/-\ c^1/-$	20262	a1			b2		c4
				20263	a1		b1	b2		c4
				20264	a1	a2			c1	
		18864	$a^1/-\ b^1b^2\ c^3c^4$	20269	a1		b1			c3 c4
				20270	a1	a2	b1			c4
				20271	a1	a2	b1	b2		
				20272	a1		b1			c3 c4
				20273	a1		b1			c3 c4
				20275	a1	a2	b1	b2		c4
2445	$a^2/-\ b^1b^2\ c^1c^4$	2475	$a^1/-\ -/-\ c^4/-$	20286	a1	a2	b1			
				20290	a1		b1		c1	
		18879	$a^2/-\ b^1b^1\ (c^1)c^2c^3$	20310	a1	a2	b1		c1	c3
				20312		a2	b1	b2	c1	c4
				20313		a2	b1			c3
				20315			b1		c1	
				20316			b1		c1	

Table 77 (Continued)

Parents - P_0				Offspring - F_1						
Sire no.	Deduced genotype[a,b]	Dam no.	Deduced genotype[a,b]	Wingband no.	Phenotypic test outcome[b]					
2451	$a^1/-\ b^1b^2\ c^1/-$	2486	$a^1/-\ b^1b^2\ c^1c^4$	20318	a1		b2	c1		
				20320	a1	a2	b1			c4
		18728	$a^1a^2\ b^1b^1\ c^1c^3$	20324	a1		b1	b2	c1	
				20325	a1		b1			c3
				20326	a1	a2	b1		c1	
				20327	a1	a2	b1		c1	
				20328	a1		b1		c1	
				20329		a2	b1			c3
				20330	a1		b1			c3
				20333	a1	a2	b1	b2	c1	
2500	$a^2/-\ b^1b^2\ c^1c^2$	2468	$a^2/-\ b^1b^2\ c^1c^4$	20336		a2			c1	
				20337		a2			c1	c4
				20338		a2	b1	b2		
18739	$a^1a^2\ b^1b^2\ c^1c^4$	2517	$a^2/-\ b^1b^2\ c^1c^1$	20347	a1		b1		c1	
				20348	a1		b1	b2	c1	
				20350	a1		b1		c1	
				20351		a2	b1		c1	
				20352		a2	b1		c1	

Table 77 (Continued)

Parents - P_0				Offspring - F_1					
Sire no.	Deduced genotype[a,b]	Dam no.	Deduced genotype[a,b]	Wingband no.	Phenotypic test outcome[b]				
18876	$a^2/-\ b^1b^2\ c^3c^4$	2425	$a^2/-\ b^1b^2\ c^1c^1$	20363	a1	a2	b1	c1	
				20365		a2	b1	c1	
				20367		a2	b1	(c1)	c3 c4
				20368		a2	b1	c1	c3
				20369		a2	b1	c1	c3
		2482	$a^2/-\ b^1b^2\ c^1c^1$	20371		a2	b1	c1	
				20374		a2	b1	(c1)	c3 c4
				20376		a2	b1	c1	c3
				20377		a2	b1 b2	c1	c4
				20378		a2	b1	c1	c3
18885	$a^2/-\ b^1b^2\ c^1c^1$	2450	$a^1/-\ -/-\ c^4/-$	20380		a2	b1	c1	
				20381	a1			c1	
				20382		a2	b1	c1	
				20383	a1			c1	
				20384	a1			c1	
				20385	a1			c1	
		2477	$a^1/-\ -/-\ c^1c^4$	20387		a2		c1	
				20390		a2		c1	
				20392	a1			c1	
				20393	a1			c1	
		2483	$a^2/-\ -/-\ c^1/-$	20394			b1	c1	
				20397			b1	c1	
				20401				c1	

Table 77 (Continued)

Parents - P_0				Offspring - F_1						
Sire no.	Deduced genotype[a,b]	Dam no.	Deduced genotype[a,b]	Wingband no.	Phenotypic test outcome[b]					
18885	$a^2/-\ b^1b^2\ c^1c^1$	2506	$a^2/-\ b^1b^2\ c^1/-$	20404	a2	b1	b2	c1		
				20405		b1		c1		
				20406		b1		c1		
18898	$-/-\ b^2b^2\ c^1c^4$	2466	$a^2/-\ -/-\ c^1/-$	20414	a2		b2	c1		
				20417	a2		b2	c1		
		2527	$a^1a^2\ b^1b^2\ c^1c^2$	20419	a1	a2		b2		c4
				20423	a1	a2	b1	b2	c1	c4
				20424	a1	a2	b1	b2	c1	c4
19102	$a^1/-\ b^1b^2\ (c^1)c^3c^4$	2416	$a^1/-\ b^2/-\ c^2c^4$	20429	a1			b2		c4
				20430	a1					c4
(18756)[c]	$a^1/-\ b^1/-\ c^2c^4$									
19112	$a^1/-\ b^1b^2\ (c^1)c^2c^3$	2484	$a^2/-\ b^1b^2\ c^1c^1$	20439	a1		b1		c1	c3
				20440		a2	b1		c1	
				20441		a2	b1	b2	c1	
				20442	a1	a2	b1	b2	c1	c3
				20443		a2	b1		c1	
				20444		a2		b2	c1	
				20446	a1			b2	c1	c3
				20447	a1		b1	b2	c1	c3
				20448	a1		b1		c1	c3

[c]Male 19102 died and was replaced by 18756 and 18906.

Table 77 (Continued)

Parents - P_0				Offspring - F_1						
Sire no.	Deduced genotype[a,b]	Dam no.	Deduced genotype[a,b]	Wingband no.	Phenotypic test outcome[b]					
		2515	a^1a^1 -/- c^1c^4	20449	a1		b2	c1	c3	
				20451	a1	b1		c1		
				20452	a1		b2	c1		c4
				20453	a1	b1	b2	c1	c3	
				20454	a1	b1	b2	c1		c4
				20456	a1	b1		(c1)	c3	c4
				20457	a1	b1	b2	(c1)	c3	c4
18903	a^1/- b^2/- $(c^1)c^3c^4$	2511	a^2a^2 b^1b^2 c^1c^1	20469	a1			c1	c3	
				20470		a2		b2	c1	c4
				20473	a1	a2		b2	c1	c3
		19113	a^1/- b^1/- c^1/-	20485	a1	a2		b2	(c1)	c3 c4
				20486			b1		c1	c3
				20487	a1			b2	c1	c3
				20488	a1		b1	b2	c1	c3
				20489		a2	b1	b2		
19102	a^1/- b^1b^2 $(c^1)c^3c^4$	2516	a^1/- b^1b^1 c^1c^4	20490	a1	a2	b1	b2	c1	c4
				20491	a1		b1			c4
(18906)[c]	a^2/- b^2/- c^4c^4			20492	a1		b1			c4
				20494		a2	b1		c1	c4
				20495	a1		b1	b2	(c1)	c3 c4
				20497	a1		b1	b2		c4
				20499	a1	a2	b1	b2		c4

Table 78. Allotyping test results for the F_2 - 1970

Parents - P_1				Offspring - F_2	
Sire no.	Deduced genotype[a,b]	Dam no.	Deduced genotype[a,b]	Wingband no.	Phenotypic test outcome[b,c]
20253	a^1a^2 b^1b^2 c^1c^4	20378	a^2a^2 b^1b^1 c^1c^3	14501	a1 a2 b1 b2 c1 c3 c4
				14502	a2 b1 b2 c1
				14503	a2 b1 b2 c3
				14504	a1 a2 b1 b2 c1 c4
				14505	a1 a2 b1 b2 c1 c4
				14506	a2 b1 b2 c1 c3 c4
				14507	a1 a2 b1 b2 c3
				14509	a2 b1 c3
				14510	a1 a2 b1 c1 c4
				14511	a2 b1 b2 c1
		20440	$a^2/-$ b^1b^1 c^1c^1	14512	a1 a2 b1 c1 c4
				14513	a2 b1 c1
				14514	a2 b1 b2 c1
				14515	a1 a2 b1 b2 c1 c4
				14516	a1 a2 b1 c1 c4
				14517	a2 b1 c1
				14519	a1 b1 b2 c1 c4
				14520	a1 b1 b2 c1 c4

[a]The dash (e.g., $a^1/-$) means the allele is unidentified, or the individual could be homozygous for the known allele.

[b]Genetics of the c allotypic system were questionable; c1 seemed to be the "extra" antigen.

[c]Offspring 14626 to 14675 were not allotyped for b2.

Table 78 (Continued)

Parents - P_1				Offspring - F_2	
Sire no.	Deduced genotype[a,b]	Dam no.	Deduced genotype[a,b]	Wingband no.	Phenotypic test outcome[b,c]
		20491	$a^1a^1\ b^1b^1\ c^4c^4$	14521	a1 b1 b2 c4
				14522	a1 b1 b2 c4
				14523	a1 a2 b1 b2 c1 c4
				14525	a1 a2 b1 b2 c1 c4
				14526	a1 b1 b2 c4
				14527	a1 b1 b2 c4
				14528	a1 a2 b1 b2 c1 c4
				14529	a1 b1 c4
20257	$a^1a^1\ b^1/-\ c^4c^4$	20239	$a^1/-\ b^1b^1\ (c^1)c^3c^4$	14533	a1 a2 b1 c1 c4
				14534	a1 a2 b1 c1 c4
				14535	a1 b1 c4
				14536	a1 a2 b1 b2 c1 c4
				14537	a1 b1 b2 c1 c4
				14538	a1 b1 b2 c1 c3 c4
				14539	a1 b1 c1 c3 c4
				14540	a1 b1 c4
				14541	a1 b1 c1 c3 c4
				14542	a1 b1 c1 c3 c4
				14543	a1 b1 c4
		20411	$a^1a^2\ b^2/-\ c^4/-$	14544	a1 a2 b1 c4
				14545	a1 a2 b1 c4
				14546	a1 a2 b2 c4
				14548	a1 a2 c4
				14549	a1 a2 b1 b2 c4
				14550	a1 a2 b2 c4
				14551	a1 a2 c4
				14552	a1 b2 c4
				14553	a1 b2 c4
				14554	a1 a2 b2 c4

Table 78 (Continued)

Parents - P_1				Offspring - F_2	
Sire no.	Deduced genotype[a,b]	Dam no.	Deduced genotype[a,b]	Wingband no.	Phenotypic test outcome[b,c]
		20473	$a^1a^2\ b^2/-\ c^1c^3$	14556	a1 b1 c1 c3 c4
				14557	a1 b1 c1 c3 c4
				14558	a1 a2 b1 c1 c4
				14559	a1 b2 c1 c3 c4
				14560	a1 a2 c1 c4
				14561	a1 b1 b2 c1 c3 c4
				14562	a1 c1 c3 c4
				14563	a1 a2 b2 c1 c4
				14564	a1 b1 c1 c3 c4
				14565	a1 a2 b1 c1 c4
		20490	$a^1a^2\ b^1b^2\ c^1c^4$	14566	a1 c4
				14567	a1 a2 b1 b2 c1 c4
				14568	a1 c4
				14569	a1 b1 c4
				14570	a1 b1 c1 c4
				14571	a2 b1 b2 c1 c4
20263	$a^1a^1\ b^1b^2\ c^4c^4$	20242	$a^1/-\ b^1/-\ (c^1)c^3c^4$	14572	a1 b1 c1 c3 c4
				14574	a1 b1 c1 c3 c4
				14575	a1 b1 c4
		20369	$a^2/-\ b^1b^1\ c^1c^3$	14578	a1 a2 b1 b2 c1 c4
				14580	a1 a2 b1 b2 c1 c4
				14581	a1 a2 b1 b2 c3 c4
				14582	a1 b1 b2 c1 c4
				14583	a1 a2 b1 b2 c1 c4
		20486	$-/-\ b^1/-\ c^1c^3$	14585	a1 b1 c3 c4
				14586	a1 c1 c3 c4
				14588	a1 b1 c1 c3 c4

Table 78 (Continued)

Parents - P_1				Offspring - F_2	
Sire no.	Deduced genotype[a,b]	Dam no.	Deduced genotype[a,b]	Wingband no.	Phenotypic test outcome[b,c]
20374	$a^2a^2\ b^1/-\ (c^1)c^3c^4$	20311	$a^1a^2\ b^1b^2\ c^3c^4$	14589	a2 b1 c3
				14590	a2 b1 c3
				14591	a2 b1 b2 c4 c4
		20404	$a^2/-\ b^1b^2\ c^1c^1$	14593	a2 b1 b2 c1
				14594	a2 b1 b2 c1 c3 c4
				14595	a2 b1 b2 c1
				14596	a2 b1 b2 c1
		20442	$a^1a^2\ b^1b^2\ c^1c^3$	14597	a2 b1 b2 c1
				14598	a1 a2 b2 c1 c3
				14599	a2 b1 b2 c1 c3
				14600	a1 a2 b1 b2 c3
				14601	a2 b2 c1
20376	$a^2/-\ b^1b^1\ c^1c^3$	20274	$a^1a^1\ b^1b^2\ (c^1)c^3c^4$	14602	a1 b1 b2 c3 c4
				14603	a1 a2 b1 b2 c1 c3 c4
				14604	a1 b1 b2 c3 c4
				14605	a1 a2 b1 b2 c3 c4
				14606	a1 a2 b1 b2 c1 c3 c4
				14607	a1 a2 b1 b2 c1 c4
				14608	a1 a2 b1 b2 c1 c3 c4
		20457	$a^1/-\ b^1b^2\ (c^1)c^3c^4$	14609	a1 a2 b1 b2 c1 c3
				14610	a1 a2 b1 c1 c3
				14611	b1 b2 c3 c4
				14613	b1 c1 c3 c4
				14614	b1 b2 c3 c4
				14615	a1 b1 b2 c1 c4
				14616	a1 b1 c3
				14617	b1 b2 c3

Table 78 (Continued)

Parents - P_1				Offspring - F_2	
Sire no.	Deduced genotype[a,b]	Dam no.	Deduced genotype[a,b]	Wingband no.	Phenotypic test outcome[b,c]
20476	$a^1a^2\ b^2/-\ c^4/-$	20201	$a^1a^2\ b^1b^2\ c^1c^4$	14618	a1 b2 c1 c4
				14619	a1 a2 b2 c1 c4
				14620	a1 a2 b2 c4
				14621	a1 a2 b2
				14622	a1 a2 b1 c1 c4
				14623	a1 a2 b2 c1 c4
		20241	$a^1a^2\ b^1/-\ c^1c^4$	14624	a2 b1 c1 c4
				14625	a1 a2 b1
20485	$a^1a^2\ b^2/-\ (c^1)c^3c^4$	20367	$a^2a^2\ b^1b^1\ c^1c^3$	14627	a2 b1 c1 c3 c4
				14628	a1 a2 b1 c1 c3
				14629	a2 b1 c1 c4
				14630	a2 b1 c3
				14631	a2 b1 c4
				14632	a2 b1 c3
				14633	a2 b1 c1 c3 c4
20492	$a^1a^1\ b^1b^1\ c^4c^4$	20275	$a^1a^2\ b^1b^2\ c^4/-$	14638	a1 b1 c4
				14639	a1 b1 c4
				14640	a1 a2 b1 c4
				14641	a1 b1 c4
				14642	a1 a2 b1 c4
20495	$a^1/-\ b^1b^2\ (c^1)c^3c^4$	20264	$a^1a^2\ -/-\ c^1/-$	14643	a1 a2 c1 c4
				14645	a1 a2 b1 c1 c3
				14646	a1 c1 c3 c4

Table 78 (Continued)

Parents - P_1				Offspring - F_2	
Sire no.	Deduced genotype[a,b]	Dam no.	Deduced genotype[a,b]	Wingband no.	Phenotypic test outcome[b,c]
20499	$a^1a^2\ b^1b^2\ c^4c^4$	20240	$a^1a^1\ b^1b^1\ (c^1)c^3c^4$	14647	a1 b1 c1 c3 c4
				14648	a1 b1 c4
				14649	a1 b1 c4
				14650	a1 b1 c4
				14651	a1 b1 c4
		20243	$a^1a^1\ b^1b^2\ (c^1)c^3c^4$	14653	a1 b1 c1 c3 c4
				14654	a1 a2 b1 c4
				14655	a1 a2 b1 c4
				14656	a1 a2 b1 c1 c3 c4
				14657	a1 b1 c3 c4
				14658	a1 b1 c4
		20254	$a^1a^2\ b^1b^2\ c^1c^4$	14660	a1 a2 b1 c4
				14661	a2 b1 c1 c4
				14662	a2 c1 c4
		20269	$a^1a^1\ b^1/-\ c^3c^4$	14666	a1 a2 b1 c4
				14667	a1 b1 c3 c4
				14668	a1 a2 b1 c3 c4
				14669	a1 b1 c4
				14670	a1 c4
				14671	a1 b1 c3 c4
		20272	$a^1a^1\ b^1b^1\ c^3c^4$	14672	a1 b1 c4
				14673	a1 a2 b1 c4
				14674	a1 a2 b1 c4
				14675	a1 a2 b1 c4